SEP 1 = 1931

Volume XXXII September, 1931 Number 3

SOIL SCIENCE

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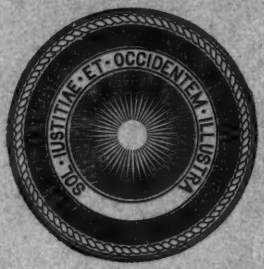
PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
Baltimore, Maryland, U. S. A.

Made in United States of America

SOIL SCIENCE



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THE USE OF HYDROGEN PEROXIDE FOR ESTIMATING HUMIFICATION

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Received for publication January 3, 1931

The work described in this note was carried out some years ago, in order to estimate the value of the 6 per cent hydrogen peroxide method for determining "degree of humification" in soils or decomposed organic materials. Clearly if a method is to effect even "an approximate separation between humified and unhumified matter" (4) the reagent used should have the minimum of action on unhumified material. In the original description of the method (4) it was shown that cellulose and "crude fiber" from hay, straw, and wood were negligibly attacked, but that washed hay and straw suffered appreciable loss (17.8 and 11.5 per cent respectively). Since in point of fact it is not "crude fiber" but whole plant material that is decomposed in the formation of humus, it was thought necessary to have more information concerning the extent to which non-humified plant materials are affected by the 6 per cent peroxide reagent before it was used for comparing the humification of materials of different origin. The results were so discouraging to the adoption of the method for quantitative purposes that the work was carried no further.

Recently, however, in a critical study of methods for determining the "humified" portion of the soil organic matter, Waksman and Stevens (5) examined the 6 per cent peroxide method along with others in studying in considerable detail four materials—sound wood (finely ground), rotted wood, forest soil, and lowmoor peat soil. After comparing the action of hydrogen peroxide and chlorine dioxide on certain fractions of these substances, those authors concluded that results with oxidizing agents could not be interpreted in terms of definite constituents of the plant residues, and so could not be used in determining degree of decomposition. They further commented on the extent (20 per cent) to which even sound wood was oxidized by the peroxide reagent. As an extension of this observation, it seemed useful to put on record the results of the present author's work: here the action of peroxide on a number of fresh plant materials had been examined, along with the extent to which it might be attributed to simple solvent action, while the further influence of soil on peroxide action was made clear.

It was found that the action of 6 per cent peroxide on all the plant materials examined was considerable, varying from 19 per cent with straw to 61 per cent with mustard tops; the effect was increased in every case by mixing with soil,

with values then ranging from 21 per cent (straw) to 70 per cent (sphagnum). With most materials, however, a great part of the effect of the peroxide reagent was simply solvent action—that is to say, treatment with water alone under the conditions of the method caused considerable losses, and the extracted residues were more resistant to peroxide. With two exceptions (sphagnum, 49 per cent, and mustard, 31 per cent) none of the residues after water extraction lost more than 16 per cent of their weight under peroxide treatment.

This result might supply some justification for the use of the peroxide method —so far as its action on undecomposed material is concerned—if it were possible to assume that under natural conditions the water-soluble portions of plant materials were completely leached away by rain and played no part in "humification." But this is unlikely, and in any case the catalytic influence of the inorganic matter, in soils, would increase the action of peroxide on the insoluble plant material. Consequently, from the point of view of the action of the peroxide reagent on *undecomposed* plant material, estimates of "degree of humification" by this method can only be very approximate; and with some materials, particularly those including sphagnum, quite unreliable.

From the point of view of the action on *decomposed* plant material, the adverse verdict of Waksman and Stevens has already been noted. In a paper of which only the abstract is at present available McLean (3) examined the action of hydrogen peroxide in a different way, by studying the effect of varying concentrations on organic matter in the soil. Regular variations in the attack of peroxide on soil nitrogen and carbon were found indicating an optimum concentration of peroxide (3 per cent), at which soil organic matter was differentiated into "two categories whereby it is possible to arrive at the condition of the organic matter of a soil at any particular time." The relationship of these categories to the degree of humification of the organic matter does not seem to be sharply defined; in any case if the 3 per cent reagent were to be adopted for this determination it would seem desirable first to examine its action on a variety of undecomposed plant materials.

In discussing the use of 6 per cent peroxide for estimating degree of humification it should not be overlooked that in practice the method has given some degree of agreement with visual observations and the known history of samples (1, 2). It seems clear that decomposed substances are more attacked than fresh materials [compare also "fresh" and "rotted" wood (5)], whether the reason is largely a matter of changing chemical constitution or in part mechanical, a result of the disintegrated condition of the decomposed material. But quantitative significance cannot be attached to results obtained by the method.

EXPERIMENTAL

The procedure employed in the present work was based on that described by Glomme (1), working with forest soils.

Two grams of oven-dried material was treated in a 250-cc. beaker with 60 cc. of 6 per cent hydrogen peroxide, heated on a water bath for 15 minutes, brought

to the boiling point over a flame, and filtered while hot through a fluted filter paper. The residue was completely washed out of the beaker with hot peroxide, and further washed on the filter with boiling peroxide until visible reaction (production of bubbles) ceased. Then after being washed three or four times with boiling water, it was transferred to a tarred porcelain or silica dish, dried in an over for about 15 hours, weighed, ignited at red heat, and again weighed. In this way it was possible to estimate both "peroxide loss" and loss on ignition.

In the first series of experiments, samples of various plant materials were treated by this "standard method." In a parallel series, the materials were mixed with soil: 2 gm. of a mixture containing 20 per cent of added plant

TABLE 1

Action of hydrogen peroxide on unhumified plant materials

	PEROXIDE LOSS	PEROXIDE LOSS IN PRESENCE OF SOIL	SOLUBLE IN BOILING WATER	PEROXIDE LOSS AFTER EXTRACTION	TOTAL LOSS, BOILING WATER FOLLOWED BY PEROXIDE
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Barley straw	16.8	21	11	8 [9]*	19
Hay	16.9	23	10	4 [5]	14
Larch needles	18.3	29	14	9 [10]	23
Beech leaves	21.6	27	15	8 [9]	23
Beech leaves, autumn	25.1	38	3	15 [15]	18
Pine needles	29.7	38	16	11 [13]	27
Lucerne tops	30.0	41	26	3 [4]	29
Oak leaves	30.5	38	21	5 [7]	26
Pasture grass	32.0	35	22	4 [5]	26
Oak leaves, autumn	34.8	44	13	14 [16]	27
Fungus mycelium	46.6	60	35	9 [14]	44
Sphagnum moss	48.3	70	5	46 [49]	51
Mangold leaves	48.3	67	41	5 [9]	46
Mustard tops	60.7	65	31	22 [31]	53

* Figures in brackets are expressed as per cent of insoluble organic material.

To these values may be added those (already quoted) obtained by other investigators who examined undecomposed plant materials: "peroxide loss" of straw, 11.5 per cent; hay 17.8 per cent; finely ground chestnut wood 20.1 per cent.

material was used, and the loss in weight of the plant material was obtained by difference, the behavior of the soil alone under the standard method being first ascertained. In this series it was found that ordinary filter paper was weakened by hot peroxide in the presence of soil, and hardened paper (Whatman 50) was used. In another series, the "standard method" was foliowed except that distilled water was used throughout instead of hydrogen peroxide, the final volume of filtrate being brought to the same level as in the standard method. The residue, after being dried and weighed, was then treated with peroxide by the standard method.

The materials examined were taken as typical of those which play a consider-

able part in the production of humus in different kinds of soil; for forest soils, were chosen pine and larch needles, and the leaves of oak and beech (both green leaves and recently fallen autumn leaves); for arable soils, barley straw, mangold leaves, and lucerne and mustard tops; for grassland, hay and young pasture grass were taken. Sphagnum moss was also examined, as being a major component of many peats; and the stems of the fructifications of a fungus, *Lepiota procera*, whose mycelium represents another material that contributes to "humus" formation. The samples were dried in a warm room, roughly ground in a hand mill, sieved (3 mm.), and oven dried for 20 hours. The soil used in one series of experiments was a Rothamsted (clay loam) soil poor in organic matter; it was similarly sieved and dried.

The results obtained are summarized in table 1. They are expressed as per cent of organic matter, taken as equal (for the purpose of this method) to the loss on ignition. The column "peroxide loss" expresses the loss in weight through treatment with 6 per cent hydrogen peroxide, which has been called the "degree of humification" in studies of decomposed materials. The values in this column are means of two or more determinations, which generally agreed closely; the greatest difference was under 2 per cent.

It is tempting to discuss these results in detail, especially in relation to what is known of the chemical composition of the different materials. High nitrogen substances after water extraction, for example, are particularly resistant, a result which may be compared with McLean's finding (3) of a resistant form of nitrogen in the soil organic matter. The measurements, however, are highly empirical; in addition to minor sources of inaccuracy, the results would be affected by changes in factors such as fineness of grinding or stage of growth of the material used. Their purpose was simple to supply information bearing on the use of the "6 per cent peroxide method," and from this point of view the results have been reviewed in the introduction.

SUMMARY

A number of undecomposed plant materials have been treated by the 6 per cent hydrogen peroxide method as used for estimating "degree of humification."

The action of the peroxide on these materials was far from negligible and was still greater in the presence of soil.

With the majority of the materials examined, simple solvent action was responsible for most of the loss under peroxide treatment, although there were notable exceptions.

From the point of view of its action on undecomposed materials the 6 per cent peroxide method can give only approximate results, and it is inadvisable to use it for comparing materials of widely differing origin.

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THE ALCOHOL METHOD FOR DETERMINING MOISTURE CONTENT OF SOILS

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Received for publication January 31, 1931

In previous preliminary communications (1, 2) the alcohol method was proposed as a rapid and accurate method for determining the moisture content of soils. It was shown that by means of methyl alcohol the moisture content of soils could be determined almost as accurately as by the drying oven method, and the time required for such a determination was short, ranging from about 5 minutes in the case of sandy soils to about 12 minutes for the heaviest clays. It was strongly emphasized that the accuracy and success of the method depends upon the alcohol coming into intimate contact with the entire soil mass and in order that this condition may be attained it is essential that the soil be thoroughly dispersed. To quote, "The soil, however, should always be reduced to the particle state so the alcohol can come in contact with the soil mass."

Smith and Flint (3) undertook to determine the accuracy of the method on a large number and variety of California soils, using the drying oven method as a standard for comparison. The results they obtained go to show that in the majority of soils, especially in the clays, the alcohol method was really a failure in determining the moisture content with any degree of accuracy.

Apparently their inability to obtain satisfactory results was due to a failure to effect complete dispersion of the soil mass. As stated in the foregoing, the first prerequisite for the accuracy and success of the method is a complete dispersion of the soil so its entire mass will come into intimate contact with the alcohol. But Smith and Flint apparently did not obtain a complete dispersion of the soils.

In dispersing the soils the writer originally used an iron rod about a quarter of an inch in diameter and flattened somewhat at the end, and by means of which it was found possible to disperse rather completely almost every kind of clay in probably not more than 10 minutes.

It is very obvious, however, that in trying to disperse the soils by hand with such a rod, the personal element enters, which makes uncertain and indefinite the degree of dispersion that the soils receive under different operators; consequently it has seemed advisable to devise a mechanical means of dispersing the soils. For this purpose, the idea was hit upon of using the milk shaking

[1] Journal Article No. 59 (n.s.), Michigan Agricultural Experiment Station.

machine such as is employed in the hydrometer method for mechanical analysis. Several modifications had to be made, however, before this mechanical means of dispersion was efficient and practical. In the first place, a special arrangement had to be made so the alcohol would not evaporate during dispersion. For this purpose, a narrow-necked-pint bottle was selected. A cork was fitted and tied at the top of the stirring rod. This cork was smoothed down so it would fit the neck of the bottle very tightly. Then on the paddle of the stirring rod was tied by means of wire, or soldered, a small steel spring such as those used with oil wicks on electric motors. This spring proved effective as an aid to dispersion. It would break up a ball of clay almost instantly, whereas without this spring the ball of soil would be stirred around and a long time would elapse before it was broken up or dispersed. The spring really adds enormously to the efficiency of dispersion of the outfit. The spring, however, should not be too long and rub on the bottle. This complete dispersion outfit is shown in plate 1.

The technique that was finally adopted for determining the moisture content of soils is as follows:

First, the hydrometer is calibrated. This is accomplished as follows: The specific gravity of pure methyl alcohol which is to be used, is first ascertained. For purpose of example, let it be assumed that it is 99.85 per cent alcohol. Exactly 75 cc. of this stock alcohol is carefully measured in a 100-cc. cylinder, 10 cc. of distilled water added to it, and the specific gravity again determined. Suppose this indicated 91.10 per cent alcohol in the mixture. The temperature must be recorded and the readings reduced to the same basis, namely 60°F. A temperature of 1°F, makes a difference of about 0.15 per cent alcohol. For temperatures above 60°F. the corresponding amount is subtracted from the percentage of alcohol indicated, and for temperatures below 60°F. the corresponding amount is added to the percentage of alcohol indicated.

When the readings are reduced to the same basis, then the reading of the alcohol which contained the 10 cc. of water is subtracted from the reading of the pure alcohol and the difference is divided into the 10 cc. of water. This gives the number of cubic centimeters of water to which each degree on the stem is equivalent. In a mixture of 75 cc. of 99.85 per cent alcohol and 10 cc. of water the standard special hydrometer gives 0.910 cc. of water for each graduation. To find the number of cubic centimeters of water in the soil sample taken the difference in specific gravity of the filtrate and pure stock alcohol is multiplied by 0.910. If 5 cc. of water is used with 75 cc. alcohol, the factor is a little less than 0.910.

Second, the general procedure for executing a moisture determination is as follows: About 25 gm. of moist soil are weighed out accurately and placed in the bottle. Exactly 75 cc. of pure methyl alcohol is poured into the bottle, the latter connected to the stirring machine and the contents stirred for about 2 minutes. In case of some soils which are seen not to have been dispersed in 2 minutes, they should be stirred a little longer. The contents of the bottle

are then poured into a filter paper on a 5-inch funnel and the filtrate allowed to drop into a 25-cc. regular graduated glass cylinder. The very first filtrate is usually turbid and consequently it is poured back into the funnel. It is well to repeat the latter process twice because it insures a clear filtrate and also serves to rinse thoroughly the graduated cylinder. Immediately after the alcohol-soil mixture is poured into the funnel, the latter is covered with a cover glass or some other means in order to prevent evaporation of the alcohol. This precaution is essential. When about 20 cc. of the filtrate is drained into the cylinder the funnel is removed and a special small alcohol hydrometer[2] is placed in the filtrate and its specific gravity and temperature are measured. From all the data at hand, the moisture content of the soil is then calculated.

EXPERIMENTAL RESULTS

In order to establish the principle with certainty that alcohol can take out all the water from a soil it is essential that the experiments be conducted under the most ridigly controlled conditions. For this purpose it was chosen to make the real test on oven-dry soils to which was added a definite and known quantity of water and then try to recover by the alcohol method the water added. This would seem to be a more logical and reliable procedure for establishing the principle, then trying to use natural moist soil, since it is difficult, and in many cases almost impossible, to take absolutely duplicate samples of natural moist soils. When the results under controlled conditions are definitely known the results obtained on natural soils can be more intelligently interpreted. The results presented in table 1, therefore, were obtained on dry soils that had passed through the 2-mm. mesh sieve. About 25 gm. of each soil was placed in the dispersion bottle, dried in an electric oven for 24 hours at a temperature of 110°C., and then placed in desiccator over sulfuric acid. When the soil had cooled 10 cc. of water was quickly added to it, and the bottle was stoppered to prevent evaporation. After sufficient time had elapsed for the soil to absorb the water the bottle was opened and 75 cc. of the stock alcohol was added. The bottle was then attached to the stirring motor and the soil mixture stirred for about 2 minutes. The alcohol mixture was then filtered, the first filtrate always being poured back, and the specific gravity determined. From the data obtained, the amount of water recovered by the alcohol was computed and compared to that added. Table 1 shows the type of results obtained from a comprehensive number of soils and artificial materials.

The results in table 1 show that the water added to the oven-dry soils is all or nearly all recovered by the alcohol. It will be seen that the amount that was not recovered is small in all cases.

These results, involving soils of maximum extremes in texture, go to establish

[2] These special alcohol hydrometers are made by Eimer and Amend. They come in pairs, one has a range of from 90 to 100 per cent alcohol and the other from 80 to 90 per cent. They are graduated into 0.5 and have a thermometer attached to them.

the principle, therefore, that the alcohol can take out practically all the water from the soil. The conclusion would seem to be justifiable, therefore, that the alcohol can be employed as a means of determining rapidly and accurately the moisture content of soils.

When the principle is definitely established that the alcohol can take out practically all the water from the soil, then the accuracy of the alcohol method for determining the moisture content of soils would depend upon the question of technique.

One of the most uncertain and difficult phases of the technique is to obtain a complete dispersion of the soil. If the soils are reduced to the particle state so the alcohol can come into contact with the entire soil mass the results in table 1 show that there is no question as to the accuracy and reliability of the method. If, on the other hand, the soils are not completely dispersed then

TABLE 1

Percentage of water recovered by methyl alcohol from water added to oven-dry soils

SOILS	AMOUNT OF WATER RECOVERED	SOILS	AMOUNT OF WATER RECOVERED
	per cent		*per cent*
Quartz sand	100.50	Parson silt loam B	99.94
Plainfield sand	100.40	Sharkley clay	100.04
Fine sand	100.50	Lake Charles clay	99.90
Miles sandy loam	100.30	Fargo clay	99.89
Kirvin fine sandy loam	99.95	Susquehanna clay	99.10
Colby silt loam	100.10	Capay clay	100.05
Grundy silt loam	99.93	Sacramento clay	99.40
Ontonagon clay B	99.95	Bentonite	99.19
Welland clay (Ontario)	99.85	Silica gel	99.40
Haldimond clay (Ontario)	99.91	Muck	99.15
Osage clay	100.05		

the results will be very inaccurate and unreliable. It seems, however, that the new device for dispersing the soils, as already described, insures complete dispersion of practically all soils. This point was thoroughly tested by using a large number of some of the heaviest clays under different moisture contents and structural condition. Some of these clays include Susquehanna clay, Capay clay, Osage clay, Sharkley clay, Welland clay, Haldemand clay, Lake Charles clay, and Fargo clay. These clays were either used in the natural moist condition or worked into paste and then into balls of different moisture contents. They were then subjected to dispersion by the stirring machine and at the end of 2 or 3 minutes they were examined to ascertain the degree of dispersion that they had undergone. It was found that in practically every case they had been reduced to the particle size.

If these clays can be completely dispersed by the stirring machine then the natural and logical conclusion from the results in table 1, is that their moisture

content can be accurately determined by the alcohol. Although this appears to be undoubtedly the case, yet, on account of the great difficulty of obtaining duplicate samples that are exactly alike in moisture content, the comparison of the moisture determination between the alcohol and drying oven methods, may not always be very close or very certain. In table 2 is shown the comparison of the moisture content, as determined by the alcohol and drying oven method, of a number of the clays. Some of these clays[3] were used in their natural moist condition; some of the other clays were worked by hand into balls of different moisture content.

The results in table 2 go to show that where the soils were worked by hand so the moisture content would be more uniformly distributed and thereby

TABLE 2

A comparison of moisture content, as determined by the alcohol and drying oven methods, of clays with natural moisture content or worked into balls by hand

SOILS	MOISTURE DETERMINED BY		REMARKS
	Alcohol	Drying oven	
	per cent	*per cent*	
Sharkey clay	33.38	34.50	Natural moist soil
Miami clay	20.46	21.96	Natural moist soil
Miami clay	25.95	25.14	Natural moist soil
Miami clay	18.45	19.64	Natural moist soil
Grundy silt loam	40.80	41.40	Worked into hard ball
Vence	22.16	22.88	Worked into ball
Susquehanna clay	27.84	28.16	Worked into ball
Asage clay	31.18	31.97	Worked into ball
Ontonagon clay	28.69	28.53	Worked into ball
Welland clay	33.05	32.85	Worked into ball
Fargo clay	35.16	36.05	Worked into ball
Osage clay	43.70	42.83	Worked into ball
Osage clay	40.20	40.55	Worked into ball

afford more uniform duplicates, the moisture content as determined by the alcohol and drying oven methods agree very closely, whereas in the case of the natural soils where uniform duplicates are difficult to obtain the agreement is not as close.

It seems almost incredible that the alcohol can reduce the moisture content of soils to almost oven-dry basis (tables 1 and 2). It is possible that the alcohol does not take out all the last traces of water in the soils, but that any traces of water that it fails to extract or replace are probably compensated by traces of material that the alcohol may dissolve out. This hypothesis would seem to be supported by the fact that in very heavy alkali soils the alcohol-water mixture dissolves the salts, and consequently the alcohol method cannot be

[3] Kindly furnished by Dr. C. F. Marbut.

used unless a correction is applied. On the other hand, in mediumly alkali soils, such as those occuring in humid regions, the method seems to work just as accurately as in the non-alkali soils. This point was tested by trying the method on certain alkali soils occurring in Iowa and the results obtained were similar to those shown in table 1.

From all the studies thus far made, there seems to be no doubt that the alcohol method, when properly used, can determine the moisture content of soils almost as accurately as the drying oven. On the other hand, to achieve this accuracy more careful technique is required in the alcohol method than in the oven-dry method.

It must be emphasized that the alcohol method for determining the moisture content of soils is not offered with the intention of replacing the oven-drying method, especially for scientific purposes. The value of the alcohol method lies in showing that the alcohol is able to reduce the moisture content to almost the oven-dry basis, in affording a method for special cases where a very rapid method is desired, and in affording a rapid and practical method for situations where other methods cannot be so easily used, such as out in the field and in field stations.

SUMMARY

The alcohol method for determining moisture content of soils very rapidly has been reinvestigated. The results obtained go to show that if the method is properly used it is accurate and reliable.

The method can determine the moisture content of soils in about 5 minutes in the case of light textured soils, and in about 12 minutes in the heaviest clays.

A new feature is introduced in the method, whereby the soils are dispersed mechanically by means of the milk shaking machine. By tying a spring to the paddle of the stirring rod, even the heaviest clay can be reduced to the particle state in a few minutes.

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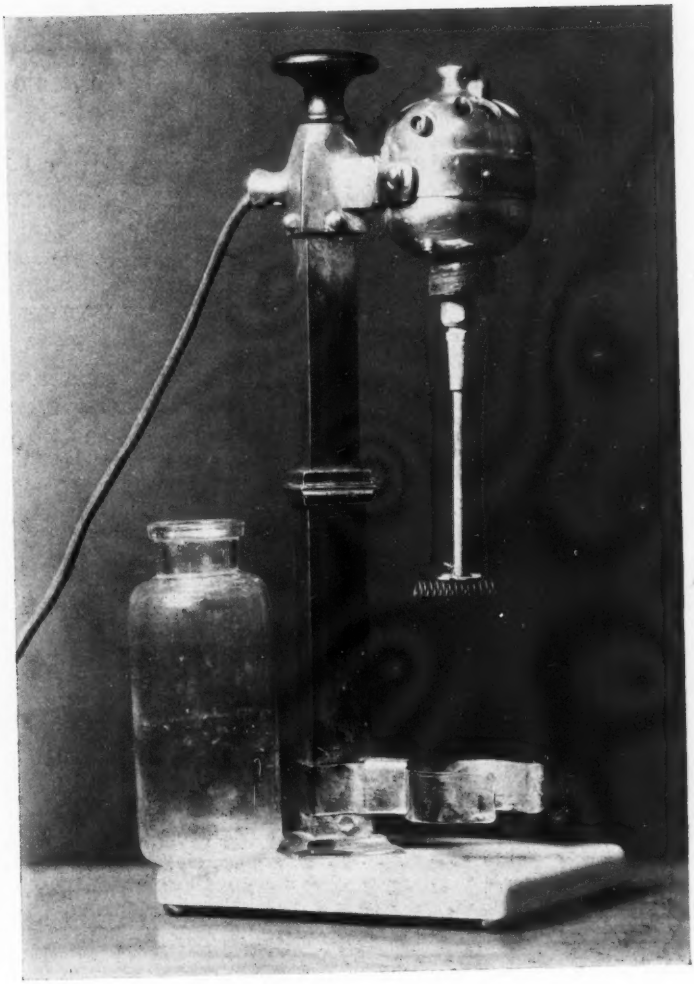
PLATE 1

Soil Dispersing Machine Showing the Spring, Cork Stopper, and Bottle

PLATE 1



THE MOISTURE EQUIVALENT AS A MEASURE OF THE FIELD CAPACITY OF SOILS

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Received for publication February 3, 1931

The moisture equivalent of Briggs and McLane (5) with the modification of the method later made by Briggs and Shantz (6) is used widely by American investigators in interpreting soil-moisture conditions. We believe that the moisture equivalent is a fairly reliable measure of the texture of the soil. A number of formulas have been suggested to indicate the relation of the moisture equivalent to the mechanical analysis (2, 6, 14, 17). All of these differ and, therefore, seem to cast doubt on the reliability of the moisture equivalent as a measure of soil texture. Since the moisture equivalent is determined by a purely arbitrary method, the differences in the formulas may be due, at least in part, to lack of uniformity in the technique of making the determinations. We have found, however, that the moisture equivalent made on 30-gm. samples according to the method previously described (23, 24) may be used as a satisfactory measure of the relative texture of soils and may, also, be used as a measure of the relative wetness of usual agricultural soils except sands (8).

Many investigators have reported tests which indicate that the moisture equivalent is a close approximation of the amount of water that the soil in the field will retain against the pull of gravity. This moisture condition of field soils is referred to in a number of ways, among which are: specific retention, maximum water-holding capacity, field-carrying capacity, normal field capacity, and maximum field or capillary capacity. We prefer the use of the term *field capacity* to indicate the amount of water held in the soil after the excess gravitational water has drained away and after the rate of downward movement of water has materially decreased, which usually takes place within 2 or 3 days in pervious soils of uniform structure and texture. As Israelsen (12) has pointed out, soils may drain slowly for a long time after irrigation, but in all of our experiments the rate of extraction of the water by plants was greater than this downward movement.

In any event, the need for a clear definition of the term used is apparent when the confusion of meaning is found in some of the reports on soil-moisture studies. In many cases the moisture contents are reported simply as certain percentages of saturation, leaving the reader in doubt as to whether complete filling of the pore space of the soil, or field capacity in the sense defined in the foregoing, is meant by saturation. In others, the soil-moisture contents are reported on the basis of the moist weight of the soil, or the percentages of moisture are given on the volume basis instead of that of the weight of the soil.

RESULTS OBTAINED BY OTHER INVESTIGATORS ON THE RELATION OF MOISTURE EQUIVALENT TO FIELD CAPACITY

In tests on loessial soils with moisture equivalents above 21 per cent Burr and Russell (7) found the average field capacity for the upper 6 feet of soil to be 91 per cent of the average moisture equivalent. Israelsen's (11) results with loams and clays which were heavily irrigated and which have moisture equivalents greater than 19 per cent, show that the maximum quantity of water held after irrigation was slightly smaller than the moisture equivalent, being about 90 per cent. Scofield and Wright (15), working with a fine sandy loam soil with moisture equivalents from about 13 to 17 per cent, concluded "that the moisture equivalent of this soil affords a fair basis for estimating its field carrying capacity after the conditions of equilibrium have become established." Mathews (13) found from tests at 17 stations in the Great Plains that the field carrying capacity of a soil is a little lower than the moisture equivalent, but bears a linear relation to it. Harding (9) has summarized the results of some 10,000 soil-moisture samples taken before and after irrigation of field plots for the purpose of comparing the moisture equivalent with the critical moisture contents of soils under field conditions of irrigation practice. He concludes "that the relationship of the soil-moisture properties to the moisture equivalent does not appear to be linear except in the case of the wilting of the crop." Harding had only 136 moisture equivalents ranging from 4.1 to 37.6 of the large number of soil samples he considered. As pointed out elsewhere (8, p. 116) serious error may be introduced if moisture equivalents are made on but a few of the samples taken. Harding's data indicate that the field capacities of the sands were higher than the moisture equivalents, whereas those of the finer texture soils were lower than the moisture equivalents.

Alway and McDole (1) from laboratory and field experiments also found the water-retaining capacities of the sands to be higher than the moisture equivalents, but those of the loams were closer to the moisture equivalents. Shaw (16) concluded from laboratory experiments that the "normal field capacity" for medium textured soils was approximately the same as the moisture equivalent.

RELATION OF MOISTURE EQUIVALENT TO PERMANENT WILTING PERCENTAGE

We have found (10, 22) that the residual moisture content of the soil at the time plants permanently wilt does not bear a linear relation to the moisture equivalent, and our results show that plants are able to reduce the moisture content of different soils to different degrees of dryness, as measured by the moisture equivalent. Furthermore, the ratios of the moisture equivalents to moisture contents at permanent wilting bear no relation to the type of soil, high and low ratios being found with sands, with loams, and with clays. The question then arises whether the moisture equivalent is a measure of the relative moisture-retaining properties of soil. Obviously, the moisture equivalent can not measure soil structure, since the original structure of the soil may be

changed greatly in preparing the samples for centrifuging and in the centrifuging process itself. We have, therefore, attempted to measure the field capacities of some of the soils we used in our experiments on the wilting of plants and have compared them with the moisture equivalents. Moisture equivalents were made as previously described (23, 24), but with better speed control of

TABLE 1

Field capacities, moisture equivalents, and relative wetness of some agricultural soils in California*

The field capacities and moisture equivalents are given as percentages on the basis of the dry weight of soil.

SOIL	LOCATION	FIELD CAPACITY	MOISTURE EQUIVALENT, AIR-DRIED SAMPLES	RELATIVE WETNESS	
				Air-dried samples, March, 1930	Oven-dried samples, August, 1930
Tehama loam	Arbuckle	15.2 ±0.13	14.1 ±0.07	1.08	1.16
San Joaquin loam	Wheatland	19.2 ±0.35	17.9 ±0.07	1.07	1.10
Columbia sand	Sheridan	21.5 ±0.45	17.9 ±0.30	1.20	1.31
Columbia silt loam	Sacramento	19.5 ±0.32	17.2 ±0.51	1.13	1.26
Columbia silt loam, 1st foot	Colusa	22.9 ±0.14	19.6 ±0.11	1.17	1.23
Columbia silt loam, 2nd foot	Colusa	16.7 ±0.33	14.2 ±0.47	1.17	1.26
Columbia silt loam, 3rd foot	Colusa	18.8 ±0.58	16.5 ±0.16	1.14	1.30
Columbia silt loam, 4th foot	Colusa	26.6 ±0.72	25.9 ±0.28	1.03	1.15
Columbia silt loam, 5th foot	Colusa	26.3 ±0.49	26.8 ±0.25	0.98	0.96
Sacramento silt loam, 1st foot	Los Molinos	25.0 ±0.33	20.0 ±0.12	1.25	1.34
Sacramento silt loam, 6th foot	Los Molinos	24.3 ±0.29	23.3 ±0.35	1.04	1.11
Stockton clay adobe	Pleasant Grove	21.2 ±0.36	22.1 ±0.21	0.96	0.99
Tehama clay	Zamora	21.4 ±0.40	20.4 ±0.34	1.05	1.09
Farwell silt loam	Chico	23.5 ±0.09	22.9 ±0.03	1.03	1.07
Capay clay	Plainfield	25.5 ±0.17	25.7 ±0.10	0.99	1.04
Aiken loam, 1st foot	Camino	25.4 ±0.47	26.6 ±0.46	0.95	0.98
Aiken loam, 2nd foot	Camino	25.5 ±0.31	26.4 ±0.13	0.96	1.04
Aiken loam, 3rd foot	Camino	25.5 ±0.16	26.3 ±0.19	0.97	1.02
Aiken loam, 4th foot	Camino	25.3 ±0.28	25.9 ±0.35	0.98	1.04

* Relative wetness is the ratio of the field capacity to the moisture equivalent expressed decimally.

the centrifuge than was found possible with the governor supplied with the standard moisture equivalent centrifuge (19).

RELATION BETWEEN MOISTURE EQUIVALENT AND FIELD CAPACITY

In March, 1930, samples were taken in fields from which the original supply of some of the soils were taken for our studies on the wilting of plants. The location of the first place of sampling was not accurately known in every case,

hence moisture equivalents of these soils listed in the following tables do not agree in every case with the moisture equivalent of our original samples.

The samples listed in table 1 were taken close to the end of the rainy season after a rainy period that was followed by 2 days of clear weather just previous to the time the samples were taken. The surface soil to a depth of about 5 inches was removed and the samples taken from about the 5 to 10-inch depth. In some cases samples were taken to a depth of 6 feet. With the exception of the Aiken loam at Camino, all of the samples were taken from cultivated fields. Undoubtedly the compacted layer below the depth of tillage in some cases had prevented complete drainage taking place during the 2 days following the rain. The samples of Columbia sand at Sheridan and the first foot of Sacramento silt loam at Los Molinos showed some free water when taken. The Columbia silt loam at Colusa was irrigated just before the samples were taken, which probably is the reason for the high relative wetness in the upper layers of this soil. Therefore, determinations of the field capacities of the soil given in table 1, are not as accurate as those of the soils listed in table 2, which were obtained under much more carefully controlled conditions.

The samples were divided, one portion being used for the determination of the moisture content by drying for 48 hours at 110°C. and the other was air dried. Moisture equivalents were determined on both the air-dried and oven-dried samples. Six months later moisture equivalents were again run on the oven-dried samples, which had been stored in paper sacks in the laboratory. The results are listed in table 1. It will be noted that oven drying changes the moisture equivalents of some soils but not of others, which is in accord with previous observations (24). In only one instance was the moisture equivalent of the oven-dried samples higher than that of the air-dried samples. It is clear that storage of the samples for 6 months did not restore the soil to a condition which gave moisture equivalents the same as those of the air-dried samples. The average ratio of the field capacity to the moisture equivalent of the air-dried samples is 1.06 $\pm$0.023, whereas the ratio for the oven-dried samples in March is 1.11 $\pm$0.026, and for those in August, 1.13 $\pm$0.026. It is obvious, therefore, that the method of preparing the samples may affect the results of the comparisons between field capacity and moisture equivalent. Oven-drying increased the difference between the field capacity and moisture equivalent in the majority of the tests listed in table 1. In some of our later tests it was not possible to use air-dried samples, so both air-dried and oven-dried samples are included in the table to show the discrepancies that are sometimes due to this factor. We feel that the best comparison between field capacity and moisture equivalent can be made on air-dried samples. Furthermore, the uses of different quantities of soil in the moisture equivalent centrifuge cups and differences in methods of preparing the samples may lead to further discrepancies.

The data in table 1 indicate that moisture equivalents of more than 14 per cent may be used as a fair measure of the field capacities of these soils.

Where compacted layers are formed by tillage, penetration of the water applied may be slowed up to such an extent that more than 2 days must be allowed for drainage before samples are taken for field capacity determination. Burr and Russell (7), Mathews (13), and Israelsen (11) all report the field capacity of their soils to be a little lower than the average moisture equivalent.

An instance of the consistency encountered in making field capacity tests at Davis is illustrated by the following data. After a heavy rain, samples were taken throughout the portion of the soil which was wetted, the depth of the sampling ranging from 2 to 16 inches. The average moisture content was 22.7 per cent and the average moisture equivalent was 22.2 per cent, a ratio of field capacity to moisture equivalent of 1.02. Several years later another test was made in another portion of the same field from which the foregoing data were taken, and the ratio of field capacity to moisture equivalent was found to be 0.97. The plot was covered to prevent evaporation and the entrance of rain, and was left for about 2 months, and a second set of samples was obtained. The ratio again was found to be 0.97. We have shown (20, 22) that the field capacities of the loam soils at Davis are very close to the moisture equivalents.

A slightly different type of test from those just described to determine the field capacities and their relation to the moisture equivalents was employed in the fall of 1928. Plots on Yolo clay at Davis; Madera and Gridley loam near Yuba City; Fresno sandy loam near Hughson; Hanford fine sandy loam near Riverside; and Oakley fine sand at Delhi were selected. Attempts were made to obtain plots on dry soil far enough removed from growing plants to prevent losses by transpiration, but it was not possible to get dry soil at all places. The plots were square, 8 feet along each side, and were enclosed by a wooden frame. There were two plots in each test with a space of about 2 feet between them. The wooden frame was set level and backed up with soil so water could not seep away from the plots. The soil was levelled by scraping off the high spots. Eight samples, evenly spaced, were taken around the outside of each plot to ascertain the moisture content of the soil before applying water to the plots. Water was applied to the plots through a meter so that the depth of application was known rather accurately. The plots were covered with canvas to prevent loss of evaporation and several sets of samples were taken within the plots. Eight samples, evenly spaced, from each plot comprised a set. These were dried for moisture determinations and the moisture equivalent determinations were made later on the oven-dried samples. The samples were taken with the soil tube previously described (18) and which has been shown can be used for volume weight determinations of the soil (3). Since it was necessary to find the volume weight of the soil we did not wish to introduce errors into this determination, as well as into that of the moisture content, by subdividing the samples to obtain a portion for air drying for the moisture equivalents. The results of the tests are given in table 2.

A trench was dug across the plots in three of the experiments after the last

TABLE 2

The relative wetness of five California soils before and after irrigation*

LOCATION	SOIL	DEPTH OF WATER APPLIED	TIME SAMPLED	RELATIVE WETNESS OF SOIL			
				Depth in inches			
				0–12	12–24	24–36	36–48
		inches					
Davis	Yolo clay	8	Before irrigation	0.47	0.66	0.79	0.88
			3 days after	0.85	0.81	0.97	0.98
			6 days after	0.92	0.82	0.97	1.00
			9 days after	0.88	0.78	0.97	0.97
		12	Before irrigation	0.47	0.65	0.82	0.92
			3 days after	0.95	0.94	1.02	1.08
			6 days after	1.01	0.94	1.08	1.10
			9 days after	0.97	0.91	1.01	1.06
Yuba City	Madera and Gridley loam	4	Before irrigation	0.22	0.41	0.50	0.53
			2 days after	1.06	0.73	0.52	..
			3 days after	0.97	0.64	0.51	..
			4 days after	0.95	0.64	0.49	..
		8	Before irrigation	0.22	0.37	0.49	0.52
			2 days after	1.06	0.93	0.76	0.64
			3 days after	1.02	0.94	0.83	0.63
			4 days after	0.99	0.85	0.78	0.65
Hughson	Fresno sandy loam	4	Before irrigation	0.31	0.31	0.34	0.39
			2 days after	1.42†	0.68	0.52	0.33
			6 days after	1.11	0.71	0.39	0.37
		6	Before irrigation	0.41	0.45	0.54	0.61
			2 days after	1.30†	1.20	0.57	0.61
			6 days after	1.18	1.03	0.76	0.62
Riverside	Hanford fine sandy loam	3	Before irrigation	0.50	0.55	0.60	0.59
			2 days after	1.38	0.88	0.54	0.59
			4 days after	1.21	1.03	0.65	0.62
			5 days after	1.13	0.99	0.77	0.73
		6	Before irrigation	0.38	0.48	0.53	0.56
			2 days after	1.34	1.26	1.09	1.09
			4 days after	1.21	1.15	1.04	1.03
			5 days after	1.24	1.16	1.05	1.11
Delhi	Oakley fine sand	3	Before irrigation	0.74	0.86	0.96	0.96
			2 days after	1.72	1.47	1.15	0.96
			3 days after	1.67	1.48	1.28	0.96
			6 days after	1.38	1.38	1.32	0.96
		6	Before irrigation	0.78	0.88	0.90	0.81
			2 days after	1.93	1.99	2.06	1.26
			3 days after	1.54	1.64	1.88	1.39
			6 days after	1.54	1.38	1.54	1.26

* Relative wetness is ratio of moisture content to moisture equivalent expressed decimally.

† Surface soil was saturated.

set of samples was taken. The line of demarcation between the wet soil and the dry soil was mapped and samples were then taken at points within and without the wetted areas. The results are indicated in figures 1 to 6. The position of the small circles indicate the place of sampling and the numbers are the ratios of the moisture contents to the moisture equivalents made on the oven-dried samples. They are the relative wetness of the soil (8) but are given as decimals and not as percentages. These data, together with those in table 2,

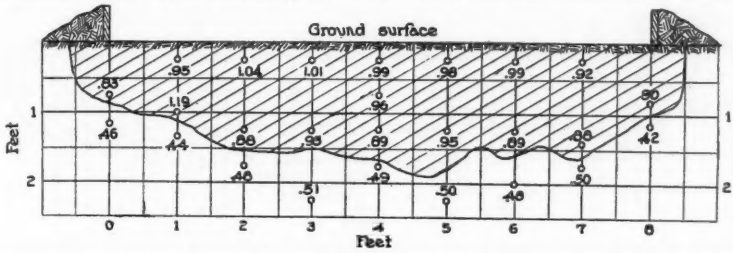

FIG. 1. FIELD CAPACITY PLOT AT YUBA CITY ON MADERA AND GRIDLEY LOAM 4 DAYS AFTER A DEPTH OF 4 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

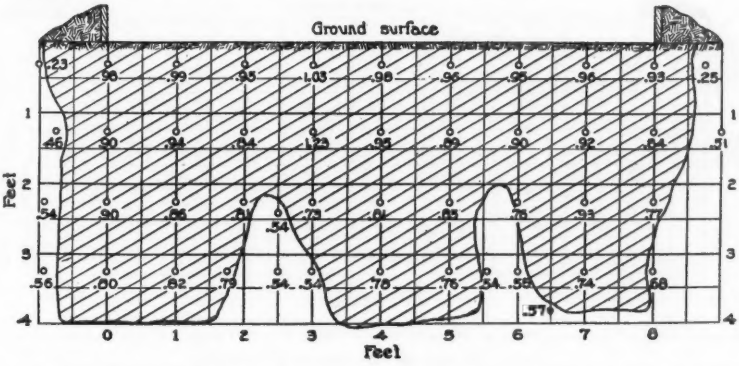

FIG. 2. FIELD CAPACITY PLOT AT YUBA CITY ON MADERA AND GRIDLEY LOAM, 4 DAYS AFTER A DEPTH OF 8 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

show that the moisture equivalent of the Yolo clay and Madera and Gridley loam is close to the field capacity, but the moisture equivalents of the sandy soils, Oakley sand, Fresno sandy loam, and Hanford fine sandy loam are less than the field capacities. The relation of the field capacities to the moisture equivalent of some of these soils as shown by regular sampling in orchard plots throughout the growing season has been discussed in a previous publication (10).

Our results showing the relation between the moisture equivalent and the field capacity agree with the findings of Alway and McDole (1) and with those of Harding (9) regarding sandy soils, but not with his results on the finer textured soils. Beckett, Blaney, and Taylor (4) report the field capacities of their soils based on a large number of soil-moisture samples and a limited number of moisture equivalents which make the comparison less exact than in the case where a moisture equivalent is made on every sample. Their results

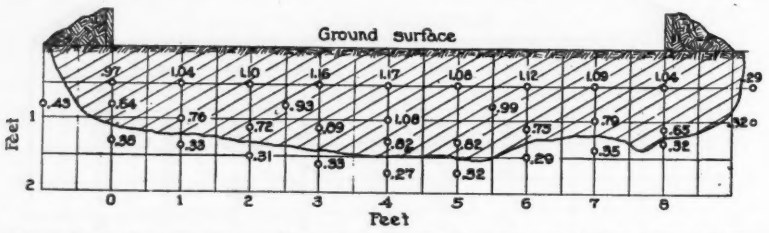

FIG. 3. FIELD CAPACITY PLOT AT HUGHSON ON FRESNO SANDY LOAM, 6 DAYS AFTER A DEPTH OF 4 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

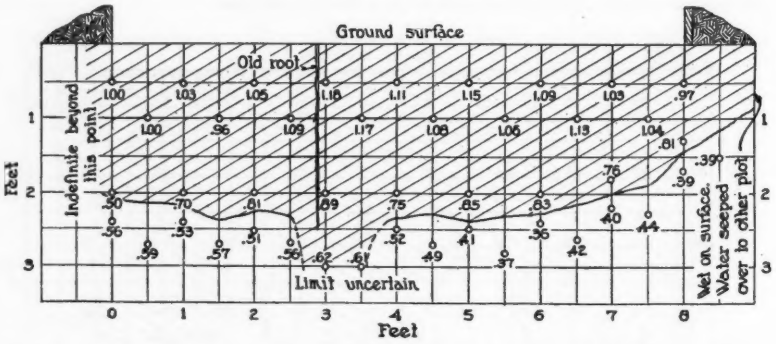

FIG. 4. FIELD CAPACITY PLOT AT HUGHSON ON FRESNO SANDY LOAM, 6 DAYS AFTER A DEPTH OF 6 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

in three different orchards on Sierra sandy loam were as follows: moisture equivalent 9.2, field capacity 11.3; moisture equivalent 11.0, field capacity 14.0; moisture equivalent 13.7, field capacity 13.5 to 15.0. The plots on Sierra loam had a moisture equivalent of 9.9 and field capacity of 12.0. The Holland sandy loam had a moisture equivalent of 14.7 and field capacity of 14.1 Here again the field capacities of the sandy soil are higher than the moisture equivalents, whereas in the fine textured soils the field capacities and the moisture equivalents are approximately equal.

INFLUENCE OF SOIL STRUCTURE ON THE RELATION OF THE MOISTURE EQUIVALENT TO THE FIELD CAPACITY

Among the factors that may influence the field capacity, and hence, the relationship between the moisture equivalent and the field capacity of a soil

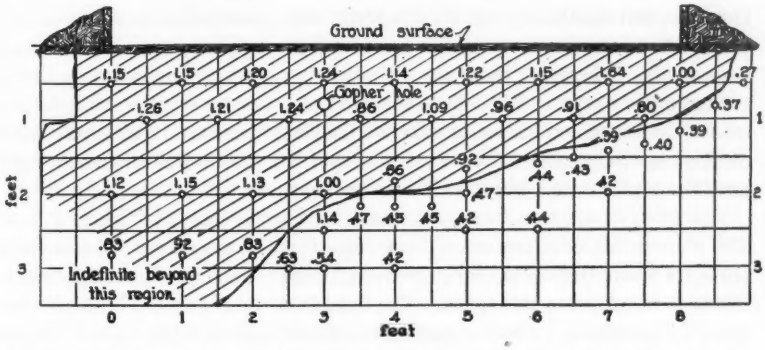

FIG. 5. FIELD CAPACITY PLOT AT RIVERSIDE ON HANFORD FINE SANDY LOAM 5 DAYS AFTER A DEPTH OF 3 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

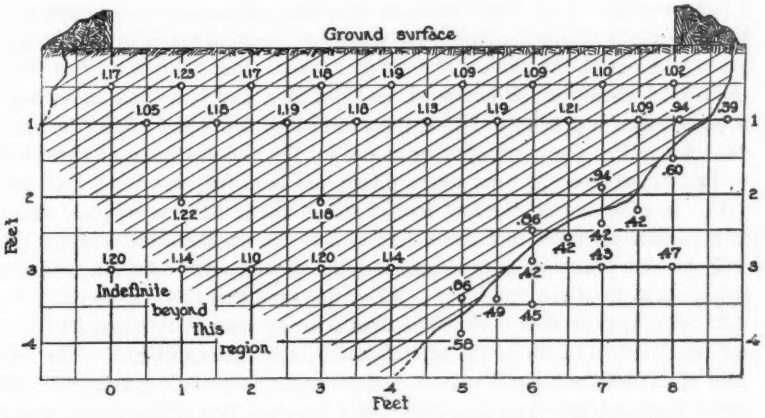

FIG. 6. FIELD CAPACITY PLOT AT RIVERSIDE ON HANFORD FINE SANDY LOAM 5 DAYS AFTER A DEPTH OF 6 INCHES OF WATER WAS APPLIED

The hatched area represents the wetted portion of the soil and the numbers are the relative wetness, or the ratio of moisture content to the moisture equivalent.

are: soil texture, depth of soil column, depth to water table, temperature, amount and kind of dissolved material in the soil water, and structure of the soil. Alway and McDole (1) have shown that continuity of texture is essential for approximately uniform distribution of water in the soil. Our experi-

ments were concerned only with discontinuities in soil structure. They showed that discontinuities in structure have such a decided effect on the field capacity that the moisture equivalent often may not be taken as a measure of the field capacity.

The Madera and Gridley loams at Yuba City, the Fresno sandy loam at Hughson, and the Oakley fine sand at Delhi were underlaid at a depth of between 4 to 6 feet with compacted layers of soil through which water permeated very slowly. Therefore, there was the possibility of water accumulating above the impervious layer. We believe this did not happen, except possibly in the case of the 6-inch application of water on the Oakley fine sand which may have resulted in a penetration of water to this layer, and downward movement may have been intercepted, but even this is questionable.

The effect of soil structure on the field capacity is shown in figures 1 to 6. The plots on Hanford fine sandy loam (figs. 5 and 6) were about 30 feet from the outer row of trees in an orange grove and close to a road. The tillage implements were turned in this space. The right hand side of figures 5 and 6 were toward the orchard. The compacting of the soil just below the depth of tillage was greater on this side than on the other. Consequently the water moved downward more rapidly on the left than on the right side, as is indicated in the figures. The volume weight or apparent specific gravity of the Hanford fine sandy loam from 0 to 12 inches deep was found to be 1.46, while the next lower 2 feet were each 1.34, which indicates that the surface soil was compacted. Volume-weight determinations were made on samples taken in the orange grove adjacent to the plots. The samples taken under the trees 2 feet from the trunks where the soil had not been tilled for many years had an average apparent specific gravity of 1.30 in the 0 to 12-inch depth and 1.34 in the 12 to 24-inch depth. The corresponding values for the samples taken at about the mid-point between the rows where tillage had been frequent were 1.47 and 1.33. In other words, the first foot of soil in the tilled area weighed about 10.5 pounds per cubic foot more than that in the untilled area, whereas the weights were about equal in the second foot of soil. At Delhi the apparent specific gravity of the first foot of Oakley fine sand was 1.54, the second foot, 1.40, and the third foot, 1.44. The Fresno sandy loam at Hughson had 1.47 for the first foot, 1.36 for the second, and 1.38 for the third foot. The first foot of Madera and Gridley loam had an apparent specific gravity of 1.30, the second and third foot each had 1.32. It seems that tillage on the sandy soils has greater effect in packing the soil than on clays. Obviously, volume-weight measurements made on the surface foot of some soils may not indicate the volume-weight of the deeper layers. The compacting of the soil in the first foot of the sandy soils may be a contributing cause for the high ratios for this depth of soil shown in table 2 and in figures 3 to 6. The slow rate of percolation on the Fresno sandy loam was particularly noticeable. The plots in this soil were irrigated on the afternoon of October 3. The first set of samples were taken on the morning of October 5, two days later, and the first

foot of soil was saturated. Drainage of free water out of the first foot continued until October 9.

A further illustration of the effect of soil structure on the downward movement of water is shown in figure 2. The irregular outline of the wetted area in the plot, to which a depth of 8 inches of water was added, is due to lens shaped masses of dense soil projecting upward from the hardpan layer. The low ratios of field capacity to moisture equivalent between and near these dry masses of soil probably are due to dry soil encountered in driving the soil-sampling tube into the soil, although these dry plots were not apparent on the face of the trench when the samples were taken. Somewhat the same condition was noticed in sampling the Yolo clay plots at Davis to which a depth of 8 inches was applied. The average ratio for the first foot, 9 days after irrigation, was only 0.88, and that of the second foot, 0.78, whereas the third and fourth feet were fully wetted as indicated by the ratios of 0.97. Some of the samples from the first 2 feet in this plot had ratios from 0.93 to 1.03 and our notes showed them to be marked "wet," but others were as low as 0.63 and were marked "dry." We believe that the failure to wet all of the upper 2 feet, while the lower layers were raised to the field capacity, even though the amount of water applied was large, was due to spots of compacted soil. The plot to which 12 inches of water was applied had the soil wetted throughout the entire depth sampled. Apparently the greater depth of water, in the latter case, resulted in holding the water on the plot long enough to allow the water to seep into the dense soil masses. The plots at Davis, which were irrigated on October 30, were located in a field which had grown a crop of barley during the previous winter and early summer. The field was not irrigated. In the past the field had been plowed and tilled when the soil was wet with resulting formation of "plow sole."

DISCUSSION AND CONCLUSIONS

The data presented here together with those of other investigators indicate that the moisture equivalent is a close measure of the field capacities of fine textured soils but not always of sandy soils. Our experiments show that the moisture equivalent can be used to indicate the field capacities of deep, drained soils with no decided changes in texture or structure, with moisture equivalents ranging from about 30 per cent down to about 12 or 14 per cent. We have not made field capacity tests with soils of finer texture than that indicated by a 35 per cent moisture equivalent. Below from 12 to 14 per cent, the moisture equivalent seems to be less than the field capacity. Inasmuch as the structure of the soil has also been shown to influence markedly the field capacity, this factor must likewise be considered in using such measurements.

The moisture equivalent seems to give a single-value determination closely related to texture, at least for the fine textured soils, in spite of the fact that the structure of the sample used in making the moisture equivalent determination may be changed from that found in the field.

Our results with the wilting of plants (10) have shown that the residual moisture content at permanent wilting, which we call the "permanent wilting percentage" does not bear a linear relation to the moisture equivalent. The ratios of moisture equivalent to permanent wilting percentage for more than 60 soils ranged from 1.4 to 3.8. Both high and low ratios were found with sands as well as with clays. It has been suggested that the moisture equivalent cannot be used as a measure of the relative moisture-holding property of soil. Our results clearly indicate, however, that it is a close measure of the field capacity of the fine textured soils, and hence, it may safely be assumed that it is a relative measure for these soils. The ratios of moisture equivalent to permanent wilting percentage for our soils with moisture equivalents of less than 12 per cent ranged from 1.51 to 3.60 with an arithmetical mean of 2.55. The ratios of the field capacities of these sandy soils, if they were known, to the permanent wilting percentage probably would be higher than those just mentioned. Certainly, the use of the field capacity instead of the moisture equivalent as a measure of the relative water-holding power of the soil would increase the magnitude of the ratios. The average of these ratios would be much higher than the commonly used 1.84 ratio, if field capacities instead of moisture equivalents for these coarse textured soils were used.

The data given in table 2 indicate that the moisture content of the soil 2 or 3 days after irrigation, which was as close to the time of irrigation as samples could be taken, does not change materially in the next few days. Apparently there was downward movement from the second to the sixth day in the plots in Fresno sandy loam and in the Oakley fine sand plots. The compacted soil in the first foot of these plots, as is indicated by the high apparent specific gravities, probably caused water to accumulate above the plow sole and impede its downward movement. Of course, growing plants would begin to use water soon after it was applied to the soil and, as we have pointed out before, all of our tests in cropped fields show that the extraction of moisture by the plants is rapid enough to prevent appreciable downward movement after the first 2 or 3 days. We feel, therefore, that there is a rather definite soil-moisture content which if measured within 2 or 3 days after a rain or an irrigation may be assumed to be the field capacity, if there are no discontinuities in structure or texture present and in the absence of a water table. Furthermore, the moisture equivalent gives a fair measure of the fine textured soils, but not necessarily of the sands.

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THE EFFECT OF DRYING AND ULTRA-VIOLET LIGHT ON SOILS[1]

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Received for publication January 6, 1931

It has been shown by numerous investigators that the drying or partial sterilization of soils may have marked effects upon their physical and chemical properties, as well as upon the crop producing power of these soils. The work herein reported was undertaken in 1927 to determine the relative effects of drying and treatment with ultra-violet light on the behavior of soils. Separate portions of a fine sandy loam soil were dried in the sunshine in the open, in sunshine under glass, in the shade, and in the oven at 100°C. Portions of soil dried in the sun and those dried in the shade as well as undried soil were treated with ultra-violet light. In treating with ultra-violet light the soil was spread out in a thin layer under a mercury vapor lamp and then at intervals of a few minutes a thin layer was removed from the upper surface with a straight edge thus exposing a fresh layer of soil. At frequent intervals the whole mass was mixed and the exposure continued. The ultra-violet light treatment of 2 kilos of soil varied from 15 minutes to 3 hours. The results reported in this paper were for the 2-hour treatments. These treated soils were compared in the laboratory and greenhouse.

BACTERIAL ACTIVITY

The ammonia content of the soil showed a slight initial increase, which was greater where the drying or treatment with ultra-violet light or a combination of the two had been most severe. After about 7 days the ammonia content decreased to a very low point.

The nitrate content of the soil was affected in the opposite direction to the accumulation of ammonia. The nitrate content became reduced immediately after treatment and then gradually increased for a period of several weeks. The average nitrate content as indicated by seven determinations over a period of 97 days following treatment showed a decided increase for the drying treatments and a still further increase where ultra-violet light was used in addition to the other treatments. A summary of these results is shown in table 1.

The nitrate-producing organisms seem to have been injured more by the treatments than were the ammonifying organisms, hence the rise in ammonia

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immediately after treatment, followed by a decrease as the nitrifying organism increased the nitrate content of the soil.

The total number of bacteria as determined on an agar media was reduced from 5,450,000 per gram in the untreated soil to about 3,170,000 in the soil treated with ultra-violet light, and to about 400,000 where dried in the sunshine. The ultra-violet light treatment, in addition to drying, reduced the numbers only slightly more than the drying treatments alone.

TABLE 1

Average nitrate content of soils during 97 days after treatment with ultra-violet light

NUMBER	TREATMENT	NO_3	INCREASE DUE TO ULTRA-VIOLET LIGHT
		p.p.m.	*per cent*
1	Moist soil—untreated	44.1	....
2	Moist soil—ultra-violet light	63.7	44.4
3	Dried in shade	95.6	....
4	Dried in shade and ultra-violet light	105.6	10.5
5	Dried in greenhouse	110.1	....
6	Dried in greenhouse and ultra-violet light	119.2	8.3
7	Dried in sunshine	121.5	....
8	Dried in sunshine and ultra-violet light	127.6	5.0

TABLE 2

Water-soluble calcium in soils with different treatments

NUMBER	TREATMENT	AVERAGE	INCREASE DUE TO ULTRA-VIOLET LIGHT
		p.p.m.	*per cent*
1	Moist soil—untreated	32.8	....
2	Moist soil—ultra-violet light	37.9	15.5
3	Dried in shade	45.1	....
4	Dried in shade—ultra-violet light	49.5	9.8
5	Dried in greenhouse	53.2	....
6	Dried in greenhouse—ultra-violet light	57.1	7.3
7	Dried in sunshine	59.2	....
8	Dried in sun—ultra-violet light	63.9	7.9
9	Dried in oven	86.2	....

WATER-SOLUBLE CALCIUM

The amount of water-soluble constituents of a soil have been shown by a number of investigations to be considerably increased by drying. This was found to be the case in this work and a still further increase was obtained by exposure to ultra-violet light. This increase was slight but apparently consistent. Table 2 will give the average of seven determinations made over a period of 97 days.

It may be seen from these figures that the amount of water-soluble calcium was almost doubled where the soil was dried in the sunshine and treated with ultra-violet light. The effect of the light was greatest on the undried soil and then decreased as the severity of the drying increased. This may have been because, in spite of certain precautions, there was some drying of this moist soil while it was being exposed to the light treatment.

RATE OF SETTLING

Soils having the various treatments were mixed with five parts water and stirred in a mixing machine for 3 minutes, after which they were placed in tall glass cylinders and allowed to stand. The colloidal material in the dried samples settled out much more rapidly than did that in the untreated. The effect of ultra-violet light was greater on the dried samples than on the untreated, but in every case there was a marked increase in the rate of settling of the colloidal material due to the effects of the ultra-violet light. The explanation for this effect is not exactly clear but may be due in part at least to the dehydration of the colloidal materials and possibly to a reduction of the charge on the particles, as shown by Nordensen (3) with metallic colloids, due to the ultra-violet light. The increase in soluble salt content in the treated soils is another factor that would induce more rapid settling.

GROWTH OF PLANTS

Soils having the various treatments of drying and ultra-violet light were used in a limited number of tests for growing plants in the greenhouse. Very little effect was found for the various treatments on the growth of roots or tops of red clover or alfalfa. Previous work by Duley and Metzger[3] showed only very slight effect of ultra-violet light on wheat. The beneficial effect of drying has been reported by a number of workers, particularly by Lebedjantzev (2) in Russia, who found in many cases a distinct increase in the growth on soils that had been previously dried. Little effect of ultra-violet light was found on the number of nodules produced on alfalfa. This is similar to results since reported by Albrecht and Turk (1) who found that *B. radicicola* were not killed in a layer of soil $\frac{1}{16}$ inch thick and exposed to ultra-violet light for 4 hours.

Further tests of dried soils and those treated with ultra-violet light should be made to determine more definitely the effect on plant growth, particularly since the work of Lebedjantzev indicated that drying had a favorable influence. Such an effect might reasonably be expected since the nitrate content, soluble calcium content, and certain other chemical and physical properties of the soil were shown in this work to have been materially altered. It seems quite possible that the ultra-violet radiation contained in sunlight may be sufficient to have some slight effect upon the properties of field soils.

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A SIMPLE ELECTRODIALYSIS CELL FOR THE ROUTINE DETERMINATION OF EXCHANGEABLE BASES IN SOILS

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Received for publication January 29, 1931

The application of electrodialysis to soils by Sante Mattson (11) introduced a promising line of attack in base exchange investigations. In a preliminary study he showed that there was good agreement between the quantities of bases removed by electrodialysis and by extraction with *N* ammonium chloride and 0.05 *N* hydrochloric acid.

Unfortunately the types of electrodialysis cells so far developed are too complicated and costly to form part of the routine equipment for soil investigations. Mattson used a three-compartment cell, the center one being separated from the two outside ones by parchment paper, which formed the dialyzing membrane. The soil was placed in the central chamber and platinum gauze electrodes were placed in the solution in the two outside chambers, which were provided with outlets for the withdrawal of dialysates. The dialysis was carried out with a 220 volt D.C. current with a 25 to 50 watt lamp in series, and the solutions were periodically changed. The temperature rose to nearly 50°C.

Clark, Humfeld, and Alben (5, 7, 8, 9) developed in detail the technique used in the handling of this type of cell. Minor changes in construction were made, which were found to be a distinct improvement, a lower voltage of 110 volts was used with or without a lamp in series, and a cooling system of glass grids with a continuous flow of cold water inside each of the compartments was introduced. The temperature was thereby kept between 18 and 22°C. Later (8), considerable economy was effected by using copper as the cathode, and also simplifying the platinum anode. A sheet of carbon for the anode instead of platinum also gave good results for the bases, though on the acid side the solution became turbid, and was practically black from carbon separated from the electrode by the current.

The Mattson-type of cell has been used by Rost (13) with a 220 volt D.C. and a cooling device as was used by Humfeld and Alben. McGeorge used the original Mattson cell with a lower voltage, 50 to 75 volts.

The three-compartment cell described in the foregoing, although very valu-

[1] The authors wish to acknowledge their indebtedness to Mr. L. F. Newman for the great interest he has taken in this work.

One of the authors (M. L. M. S.) wishes to express his thanks to the Government of Ceylon for a post graduate scholarship, during the tenure of which this work was carried out.

able for certain types of investigation, is not well suited for routine work. If it is not necessary to separate the soluble anions from the soil, a two-compartment cell could be used for the separation of the bases, the anode being placed in the same compartment as the soil. This arrangement eliminates the resistance of one membrane, permits the electrodes to be placed more closely together, and greatly accelerates the dialysis. Bradfield (2, 3), developed a type of cell suitable for routine work, based on the above considerations. The soil sample is placed in a clean alundum extraction thimble (12.7 x 3.2 cm.) which is supported by a perforated nickel cylindrical cathode suspended in a specially constructed glass cell with a side arm. The platinum foil anode is placed on the inside of the alundum thimble. The electrodialyzate is drawn off from the side arm of the cathode chamber, the water being maintained at a constant level inside the cell by means of a siphon connected with a storage bottle in which a constant level is maintained. After it has been started the apparatus is automatic. The electrodialysis is continued until the dialyzate remains colorless when treated with phenolphthalein. Usually a liter of dialyzate is obtained in about 4 to 8 hours. The total bases are titrated by adding excess of standard acid, and back titration with standard alkali, after which the individual bases can be determined in the solution.

The Bradfield type of cell has also been used by Wilson (17, 18, 19, 20), in a series of investigations.

Crowther and Basu (6), using the Bradfield cell, found that still larger volumes than 1 liter are sometimes required for the complete removal of the bases. The dialyzate is made too dilute by the large amount of electroendosmose through the sides of the thimble in comparison with the transport of ions. This is eliminated to a large extent in the modified cell devised by Crowther and Basu, in which the space between the electrodes is reduced to a minimum, and the whole of the soil but none of the uncovered membrane lies directly between the two electrodes. The membrane consists of a shorter and wider alundum R.84 thimble (5.5 cm. long and 3.5 cm. external diameter). Ten grams of soil is placed on the flat bottom of the thimble and covered by a filter paper. The anode is a perforated disc of platinum or gold, and rests on the soil. The thimble rests on a nickel cathode on the floor of a glass cathode chamber. A constant water level is maintained in the anode chamber. With 100 volts the temperature of the soil does not rise above 40°C. if the current is adjusted by an external resistance. Electrodialysis is continued for about 1 hour after the dialyzate ceases to give a coloration with phenolphthalein. Usually a volume of 500 to 700 cc. was sufficient for the complete removal of bases in 6 to 8 hours. Traces of calcium and magnesium carbonates deposited on the outside of the thimble and in the cathode chamber are recovered by cleaning the inside of the thimble and then boiling it with water containing a known amount of 0.1 *N* acid and again with water. The solutions are again used in turn to wash out the cathode chamber and are added to the dialyzate. The total

bases are determined by back titration as described before, and the individual bases subsequently estimated.

The type of cell described in the foregoing, though the simplest so far developed, is nevertheless too expensive for a routine apparatus, and is considerably more complicated and costly than the cell described in the following.[2]

In the type of cell described in this paper, the cost of the outfit has been reduced to a minimum, chiefly through the simplicity of the electrodes, besides eliminating the outer cathode chamber. The whole outfit is within the means of any soil investigator.

THE NEW SIMPLE TWO-COMPARTMENT CELL

The principle of the cell described in this paper is based on the type of cell developed by Basu and Crowther (6), where the soil layer is reduced to a minimum in the form of a flat thin layer parallel to, and against, which is placed the anode. The cathode is placed below the flat bottom of the dialyzing vessel. The anode chamber consists of a special type of Jena glass filtering crucible, more or less like a Gooch crucible, in which the filter disc is made from sifted powdered glass, heated to its sintering point, and without any bonding material; the filter disc[3] is permanently sealed into the Jena glass.

The grade of porosity of the diaphragm found to be most suitable was No. 4., the average size of the pores of which was 5 to 10 μ. The capacity of the vessel is 60 cc., diameter of diaphragm 4 cm., and height above diaphragm 5 cm. Since the filter plate is made of glass without any bonding material, it contains no exchangeable bases such as occur in most forms of alundum, which need preliminary treatment and are likely to absorb bases during use. The bottom of the crucible extends below the filter disc to nearly 1 cm., narrowing down slightly as shown in figure 1a. The filter disc forms the dialyzing membrane, and can be easily cleaned after use. The walls being made of glass are nonporous, therefore the endosmotic flow of water is through the filter disc only, so that the dialyzate obtained is not diluted.

The cathode consists of a circular piece of copper gauze fitted below the filter disc and close to it as shown on the diagram. This can easily be done by cutting the gauze slightly larger than the size of the filter plate, and working in the edges of the gauze closely toward the sides of the diaphragm by means of a small screw driver. Two layers of filter paper are first similarly placed between the filter plate and the copper gauze before the latter is fitted, for reasons explained later. The copper gauze is cut so that a portion of it extends below, bent as shown, so that it can be connected to the negative terminal. The connection is made through a hole pierced in the gauze through which the terminal is

[2] According to the catalogued price supplied by Messrs. Gallenkamp, six gold electrodes alone cost $45, whereas the corresponding nickel cathodes cost $17.

[3] These filters are supplied by Messrs. Gallenkamp & Co. Technico House, 17 to 29 Sun Street, Finsbury Square, London, E. C. 2., price 3/9. (See catalogue on "Fine Filtration and Ultra Filtration.")

attached in the form of a hook. After the cathode has been carefully fitted, the soil sample (10 gm. of a clay soil, or 25 gm. of a light soil) is placed in the crucible, and washed under suction with 200 cc. of 50 per cent alcohol, and

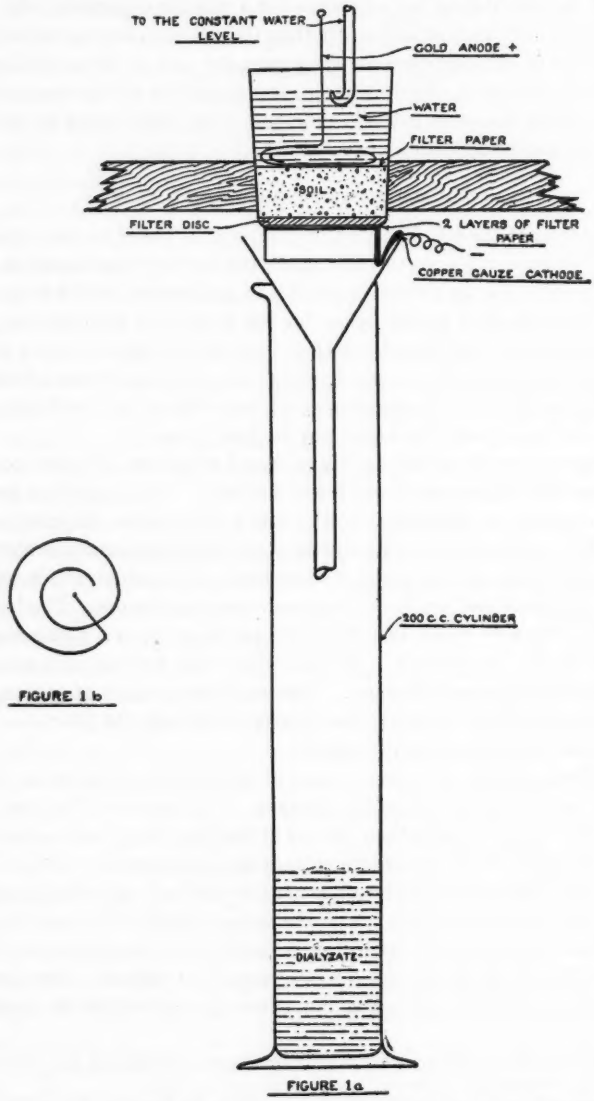

FIG. 1. *a*, DIAGRAM OF THE NEW TWO-COMPARTMENT CELL; *b*, THE ANODE USED IN THE CELL

finally with 100 cc. distilled water, to remove soluble salts and especially chlorides. This is necessary as the traces of chlorides liberate chlorine during electrodialysis, which attacks the gold wire used as the anode. The anode consists of 10 inches of pure gold wire (English S.W.G. 24, .559 mm. diameter), bent in the form of a plane spiral of three turns as shown in figure 1b, placed parallel to, and lying on, the soil. It is preferable to place a layer of filter paper on the soil, and then the gold anode above, to prevent the layer of soil being disturbed by the water. Ten gold electrodes can be made from $12 worth of gold wire. The cell, after it has been fitted and is ready for use, rests in a hole in a wooden framework. The crucible is nearly filled with water and connected to a constant water level arrangement, after which the electrodes are connected to the terminals.

When the potential difference is applied there is an endosmotic flow toward the cathode of water which carries the cations to the filter disc, when they are liberated as hydroxides at the cathode. These are continuously removed by the endosmotic stream and drip into the funnel held below, and are placed in a 200-cc. measuring cylinder. After it has been started the process is automatic, the dialyzate being continually changed in the process.

In plate 1 are shown a set of 10 cells, mounted in two rows on a wooden framework, and connected in parallel to the main terminals. The anodes are connected to the insulated wire on top, and the cathodes are connected from below to the other insulated wire. The main terminals are connected to a D.C. of 200 volts and the external resistance is adjusted by means of a lamp of 15 to 100 watts in series depending on the number of cells in the circuit. The total initial current was rarely more than 200 milliamperes, but gradually fell toward the end; during the later stage, lamps of successively lower resistance were substituted.

The constant water level consists of a Marionette's bottle which is connected to the cells by means of T pieces and regulated in the usual manner.

The dialysis is continued for 1 hour after the dialyzate ceases to give a color with phenolphthalein.

EXPERIMENTAL

Soils examined

Four soil types were used in a critical study of the cell described in the foregoing:

Oxford clay: A heavy subsoil, decalcified, from top of brick pit at Warboys, Huntingdonshire. Because of the proximity of the pit the land had not been cultivated for at least 40 years. pH 6.7.

Lower greensand: A light, market garden topsoil from Gamlingay, Cambridgeshire. pH 6.1.

London clay: Heavy clay topsoil from Purleigh, Essex. pH 6.6.

Fen peat: Peat topsoil from the Fenland of Kesteven, Lincolnshire. pH 4.3.

Methods

Ten grams of the Oxford clay, London clay, and peat, or 25 gm. of the lower greensand soils were used for electrodialysis.

All the following analyses were carried out in duplicate:

Total exchangeable bases.—The total exchangeable bases in the dialyzate were not determined by direct titration with standard acid as was done by Mattson, Bradfield, or Crowther and Basu. The technique used was slightly different, as traces of calcium and magnesium carbonates were deposited on the outside of the filter disc on the lower edges of the crucible and the funnel. Usually, especially from the peat soil, a brown deposit of iron hydroxide was formed on the filter plate. This was prevented by introducing two layers of filter paper between the gauze electrode and the filter plate as mentioned before, when this deposit, as also the traces of calcium and magnesium carbonate, were concentrated mainly on this filter paper. Instead of removing these traces with standard acid as was done by Crowther and Basu, they were removed by means of 10 per cent acetic acid. The copper gauze electrode was also left in acetic acid for a few minutes, and then washed with distilled water, the filter similarly treated, and the traces adhering to the filter plate and the edges of the crucible also were removed. The funnel and the collecting cylinders were washed in acetic acid and added to the bulk of the dialyzate, together with the other washings, which were all evaporated down on a water bath in a large porcelain dish. When the volume became small the residue was transferred to a 9-cm. porcelain dish in which the subsequent operations were carried out. When the evaporation was complete the dish was dried over a Rose burner and when dry finally ignited in a muffle furnace at a dull red heat for 5 minutes.

In this process the acetates are quantitatively converted to carbonates or oxides as shown by Rice Williams (16), and by Bray and Willhite (4). The bases were then estimated, using the details of Rice Williams. When cold, 50 cc. of 0.2 *N* hydrochloric acid was added, the solid being well rubbed in the acid with a rubber tipped glass rod. The dish was covered with a clock glass and allowed to stand over night. The contents of the dish were then filtered through a small filter paper into a 600-cc. beaker and washed with hot distilled water. When cold the excess acid in the filtrate was titrated with 0.2 *N* sodium hydroxide, using methyl red as indicator. Using standard sulfuric acid and boiling off the carbon dioxide after filtration gave the same results as in the aforementioned process, showing that when these details are used, no appreciable amount of carbon dioxide remains. The total bases were calculated in milligram equivalents from the volume of acid used.

Besides the fact that the filtrate so obtained after titration with standard alkali can be used without further treatment for the determination of the exchangeable calcium, magnesium, and potassium, the treatment is indispensable in the determination of the total bases of the dialyzate from the peat soil as it was colored brown by the humic matter.

Exchangeable calcium.—If magnesium is to be determined, the soluble silica

in the filtrate after titration is removed by evaporation to dryness and heating for 3 hours in a hot air oven below 120°C.[4] The residue was taken up in hydrochloric acid and filtered.

To the filtrate 5 gm. ammonium chloride was added, the solution heated to boiling, 1 gm. oxalic acid added, and the calcium precipitated as oxalate by Blasdale's method (15), by adding 1 per cent ammonia, and being allowed to stand 4 hours. The calcium in the precipitate was determined after washing by the volumetric permanganate method after solution in sulfuric acid.

Exchangeable magnesium.—In the filtrate from calcium oxalate the magnesium was precipitated by means of sodium phosphate and 10 per cent ammonia. After standing 24 hours the precipitate was filtered, ignited, and estimated as the pyrophosphate.

Exchangeable potassium.—The potassium was determined in the dialyzate from another cell after titration of the total bases by the volumetric-cobaltinitrite-permanganate method, using the technique developed by Milne (12). Here the advantage of the preliminary acetic acid treatment and subsequent ignition becomes apparent. This serves for the determination of the total bases, for the removal of the organic matter, and the sesquioxides for the potassium determination. No further treatment was necessary besides evaporation to a small bulk.

Very satisfactory agreement between duplicates was obtained in all the foregoing determinations.

Exchangeable sodium was not determined.

Exchangeable bases by displacement.—The same amounts of soil as were used for electrodialysis were treated with *N* ammonium acetate, in preference to *N* ammonium chloride. The advantages of ammonium acetate have been discussed by Schollenberger (14). The total bases can be determined by converting the acetates to carbonates by ignition, followed by titration as before by the methods of Bray and Willhite (4), and Rice Williams (16). The soils were leached up to a volume of 500 cc. with ammonium acetate adjusted to pH 7. The ammonium acetate was removed by volatilization on the water bath, and the total bases replaced as acetates were determined as before and subsequently the individual bases in the titrated solutions.

Hydrogen-ion concentration.—The pH values of the soils before and after electrodialysis were determined with a quinhydrone electrode and a soil-water ration of 1:2.

DISCUSSION

Exchangeable bases

The quantities of exchangeable calcium, magnesium, and potassium extracted, when the soils were electrodialyzed or leached with *N* ammonium acetate, are shown in table 1. The values are expressed in milligram equivalents per 100 gm. soil.

[4] J. W. Mellor: Treatise on Quantitative Inorganic Analysis, Vol. I.

TABLE 1

Exchangeable bases as determined by electrodialysis and by displacement with normal ammonium acetate

SOIL	MILLIGRAM EQUIVALENTS PER 100 GM. SOIL															
	1 Calcium		2 Magnesium		3 Potassium		4 Ca, Mg, K		5 Total by titration		6 Difference 4–5		7 $CaSO_4$		8 Ca, Mg, K 4–7 corrected	
	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement	Dialysis	Displacement
Lower greensand	5.39	5.49	0.99	0.89	0.50	0.59	6.88	6.97	5.70	5.37	1.18	1.60	.....	0.99	.....	5.98
Oxford clay	24.22	24.36	3.46	3.08	0.76	0.84	28.44	28.28	25.56	25.12	2.88	3.10	1.70	3.26	26.74	25.02
London clay	21.21	21.16	.....	6.66	0.92	0.82	.....	28.64	27.24	26.74	.....	1.90	.....	2.66	.....	25.98
Fen soil	38.72	36.57	1.76	1.76	0.53	0.69	41.01	39.02	37.77	35.70	3.24	3.32	2.47	5.18	38.54	33.84

A comparison of the individual bases as determined by the two methods employed shows these to be in close agreement. There is excellent agreement for calcium, whereas the agreement for magnesium and potassium is satisfactory, considering the small quantities of these bases estimated. In the case of magnesium, for the four soils examined, no discrepancies such as were recorded by Wilson (17) in the values obtained by the two methods employed are shown by our results. Wilson noted that considerably less magnesium was recovered by electrodialysis.

Similar agreement is shown in the values for the total bases by titration by the two methods, except for the slight discrepancy in the case of the Peat soil. The sum of the individual bases shown in column 4 shows similar agreement. When, however, the sum of the bases in column 4 is compared with the values for the total bases by titration given in column 5, very considerable discrepancies are noted, the values by titration being lower, as much as 12 per cent in the case of the Oxford clay. Such divergencies cannot be accounted for by experimental errors, therefore some other acid radical which does not give an oxide or carbonate on ignition must be present.

In these soils the likely radicals are sulfate, and perhaps phosphate. Such differences of the order recorded in column 6 have been noted by Rice Williams (16). In the four soils under discussion in this paper the differences were traced to sulfate. The ammonium acetate leachine liquid was evaporated and ignited, and the residue taken up in hydrochloric acid was examined for sulfate. The amounts recorded in column 7 were determined. Traces of phosphate were found in the extract of the Lower Greensand soil.

It may be noted that the amounts in column 7 are larger than the differences in column 4. This is because the values for exchangeable sodium have not been included in the sum of the bases determined individually. As it was found that sulfates are present in the soils, samples of Oxford clay and the peat soil in which the largest discrepancies were noted were again electrodialyzed, and after preliminary ignition and solution in hydrochloric acid, were examined for sulfates. The amounts found are recorded in column 7. The sulfuric acid is washed down into the basic dialyzate, possibly toward the end of the process, and reacting with the bases, lowers the titration value. Hence the value for exchangeable calcium is higher than the true figure, and the total bases by titration give the correct value for the total exchangeable bases, i.e., the S-value of Hissink.

The figures for calcium sulfate obtained by dialysis (column 7, table 1) are slightly lower than the corresponding values obtained by displacement, which is perhaps due to the fact that the dialyzates on which the sulfates were determined were obtained in 6 hours. The dialyzates on which the total titration values were determined were obtained in 14 hours with a slightly lower current and with a larger volume of water, which may have carried down more sulfuric acid.

Rate of dialysis and volume of dialyzate

Humfeld and Alben (8), using the modified Mattson cell, obtained complete dialysis in 45 hours. Rost (13), found it necessary to continue the process for

TABLE 2

Rate of dialysis, volume of dialyzate, total bases extracts, and temperature of cells, using gold wire anodes and 60-cc. cells

SOIL	TIME*	VOLUME OF DIALYZATE	TOTAL BASES IN M.E. PER 100 GM. SOIL	NUMBER OF CELLS IN CIRCUIT	TEMPERATURE	LAMP
	hours	*cc.*			*°C.*	*w.*
London clay	(a) 11.5	200	27.15	2	29	25
	(a) 7	250	26.9	3	22	25
	(b) 3	200				
London clay	(a) 8	250	27.50	3	23	25
	(b) 2	60				
	(a) 6.5	200	27.4	3	23	
	(b) 3	150				
	(a) 11	300	5.56	10	25	40
	(b) 1.5	100				
Greensand	(a) 14.5	300	5.44	10	24	
	(b) 1.5	80				
	(a) 13	380	5.88	10	25	
	(b) 1.5	150				
	(a) 14	380	25.40	10	24	40
	(b) 1	20				
Oxford clay	(a) 10	180	25.4	10	29	40
	(b) 2	50				
	(a) 12.5	450	25.60	10	29	40
	(b) 2	50				
	(a) 7.5	330	38.50	6	25	40
	(b) 2	360				
Fen peat soil	(a) 7.5	260	36.70	6	25	40
	(b) 2	560				

* (a) = Time when dialysis was complete as indicated by phenolphthalein.
(b) = Extra period for which dialysis was run.

the much longer period of 5 days. These authors do not state for comparison the results obtained by displacement methods. No data are given for the vol-

umes of the dialyzates obtained. Wilson (17), using the Bradfield two-compartment cell, continued the electrodialysis for 8 hours at an amperage of 100 milliamperes, and usually about 1 liter of the dialyzate was obtained. Except for magnesium, the values for the bases extracted by dialysis and by displacement showed close agreement. Crowther and Basu (6), using the Bradfield apparatus, found that still larger volumes than 1 liter are required for the complete removal of bases. But with their type of cell they found that a volume of 500 to 700 cc., removed in 6 to 8 hours, was sufficient for loams and clays, but 15 hours was necessary for light soils in which the initial current was very low, about 80 milliamperes.

TABLE 3

Rate of dialysis, volume of dialyzate, and temperature of cell

Results with carbon rod anode and 30-cc. cell

SOIL	TIME	VOLUME OF DIALYZATE	TOTAL BASES BY TITRATION*	NUMBER OF CELLS IN CIRCUIT	TEMPERATURE
	hours	*cc.*			°C.
Fen soil (peat)	(a) 17.5	734	37.80	8	18–23
	(b) 4	226			
	(a) 17.5	470	37.90	8	20–26
	(b) 4	130			
	(a) 17.5	222	26.5	8	22–31
	(b) 4	138			
London clay	(a) 17.5	80	25.2	8	23–33
	(b) 4	30			
	(a) 17.5	145	25.3	8	23–28
	(b) 4	60			
	(a) 17.5	100	29.2	8	22–27
	(b) 4	38			

* M.E. per 100 gm. of soil.

The results obtained with the new cell described are given in table 2. No attempt was made to measure the current passing through each cell, but only the total current passing through all the cells. The time required was dependent on the current as shown by the fact that when few cells were run in the circuit less time was required for complete dialysis. When 3 cells were run at the same time with a 25 watt lamp in the circuit, the London clay was completely dialyzed in 7 to 8 hours. A soil of similar texture and of practically the same total base content, the Oxford clay, required from 10 to 14 hours when 10 cells were in the circuit with a 40 watt lamp. With 6 cells in the circuit with a 40 watt lamp the peat soil of high base exchange capacity was completely dialyzed

in 7 hours. With clays and peat soils having a large amount of total bases, the initial current is higher than with light soils such as the Greensand, and the initial removal of the bases is very rapid. However, the current soon falls with increasing resistance, and towards the end the rate of dialysis slows down. Though actual data of fractional dialysis are not given, most of the bases were removed in the first 3 hours in the case of clays and peats. Ten cells were run at the same time, five with the Lower Greensand, and five with the Oxford clay soils, and though the initial current through the former was very small compared with the latter, dialysis was complete in both cases in nearly the same time—14 hours. The initial current passing through all the cells was never more than 200 milliamperes, and dropped toward the end.

The volume of the dialyzate varied with the texture of the soil. With heavy soils such as the Oxford and London clays, very concentrated dialyzates were obtained—dialysis was complete with 200 to 300 cc. The current was run for an extra hour, however, and in this period the flow of water increased rapidly. This result is in contrast to that of Crowther and Basu (6), who used an alundum membrane and found that the rate of flow of the dialyzate steadily decreased with time. Table 2 clearly shows this increase of flow toward the end, and the effect of permeability of the soil on the rate of flow. With the Greensand soil about 300 cc. of dialyzate was obtained, whereas from the Peat soil comparatively large volumes were obtained, sometimes as much as 800 cc.

Table 3 records the results obtained with a 30-cc. cell, and a carbon rod as anode, and with a copper gauze cathode. The carbon rod was of the ordinary uncored type used in arc lamps, and was suspended vertically in the soil. Ten grams of soil was used for the dialysis, but although only 8 cells were run in the circuit, the current passing was very small because of the high resistance of the thick soil layer in these small cells. The results obtained for the peat soils showed close agreement with those recorded in table 2, with the gold anode. Another disadvantage was the separation of fine carbon which passed into the pores of the filter plate, and would probably have rendered its life short. The time taken for complete dialysis was 17 to 18 hours.

Temperature

A unique feature of the cell described is that there is no appreciable rise in temperature of the soil. The current through each cell is small, as the total initial current with 10 cells was never more than 200 milliamperes. The temperature never rose above 29°C., and was usually between 18 and 25°C. Even when 3 cells were run, as long as the current was adjusted by a lamp, the temperature did not rise appreciably.

Hydrogen-ion concentration before and after electrodialysis

The pH values of the soils of the investigation before and after electrodialysis appear in table 4. The Peat soil, though originally very acid (pH 4.27), contained the largest amount of bases because of the high absorption capacity of

the organic complex. The final pH values after complete removal of soluble anions ranged from 3.5 to 4.0, and are in conformity with the values obtained by Bradfield (2), Wilson (17), and others.

DECOMPOSITION OF THE ABSORPTION COMPLEX

As previously noted, in all cases a brownish deposit of iron and manganese hydroxides was formed on the bottom of the filter plate, and was later concentrated on the two layers of filter paper placed between the copper gauze and the filter disc. The amount was very small with all the soils except the peat. A colorimetric estimation showed the former to be the equivalent of about 0.0005 gm. mn and an equal amount of iron. With the peat soil, however, a considerable quantity of the brown deposit was concentrated on the filter papers, consisting mainly of iron hydroxide. McGeorge (10) states: "Large amounts of iron, aluminum and manganese did not make their appearance in the dialyzate until the calcium, magnesium, sodium and potassium were all completely removed." Humfeld and Alben (8) noticed a whiteish flocculent precipitate,

TABLE 4

Hydrogen-ion concentration of soils before and after electrodialysis

SOIL	pH BEFORE DIALYSIS	pH AFTER DIALYSIS	TOTAL BASES BY TITRATION*
Lower greensand	6.07	4.08	5.70
Oxford clay	6.70	3.71	25.56
London clay	6.55	3.51	27.24
Peat soil	4.27	3.58	37.77

* M. E. per 100 gm. of soil.

apparently aluminum hydroxide, which appeared in increasing amounts as the solution tended to approach a neutral reaction, and with further dialysis and subsequent changes of solution the precipitate changed to a brown color, indicating increasing amounts of iron hydroxides, and possibly decreasing amounts of alumina. No such deposit was noted in the dialyzates obtained by the authors, except for the brown deposit on the filter papers.

This brings us to the much discussed question of the presence of iron, aluminum, and manganese in soils, as exchangeable components of the absorbing complex. McGeorge (10) tested this in detail by determining the exchange capacity of the soil before and after electrodialysis. No change in exchange capacity was observed, and he concludes that iron, aluminum, and manganese were not derived from the "zeolites," but rather were dissolved by the action of the hydrogen-ion upon the hydroxides and oxides present in the soil, and he questions the presence of these elements in an exchangeable form in soils. This question is at present under investigation by the authors, with special reference to the peat soil. The results of McGeorge indicate a highly stable exchange complex in soils.

SUMMARY

The various types of electrodialysis cells so far developed are unsatisfactory for routine work, and the cost of the apparatus is high. A simple and cheap two-compartment continuous-flow type of electrodialysis cell, well suited for routine determinations of the exchangeable bases of soils, has been described. A special type of filtering crucible is used as the anode chamber, with a filter plate made of sintered glass powder as the dialyzing membrane. The cost of the apparatus is greatly reduced by the use of a plane spiral of gold wire as the anode, and by the elimination of the outer chamber. Copper gauze is used as the cathode.

The cell is easily constructed, and has the following advantages: The soil layer is thin. There is no endosmotic flow of water through the sides, as these are of glass. The distance between the electrodes is short. The soil can be washed free of soluble salts before electrodialysis. Concentrated dialyzates are obtained. The cell can be easily cleaned. There is practically no rise in temperature during dialysis. Complete dialysis can be effected in 6 to 10 hours. A set of 10 cells complete with electrodes can be fitted up for about $20.

Results obtained with four types of soils are discussed. Very close agreement was obtained between the bases extracted by electrodialysis and those extracted by displacement with normal ammonium acetate.

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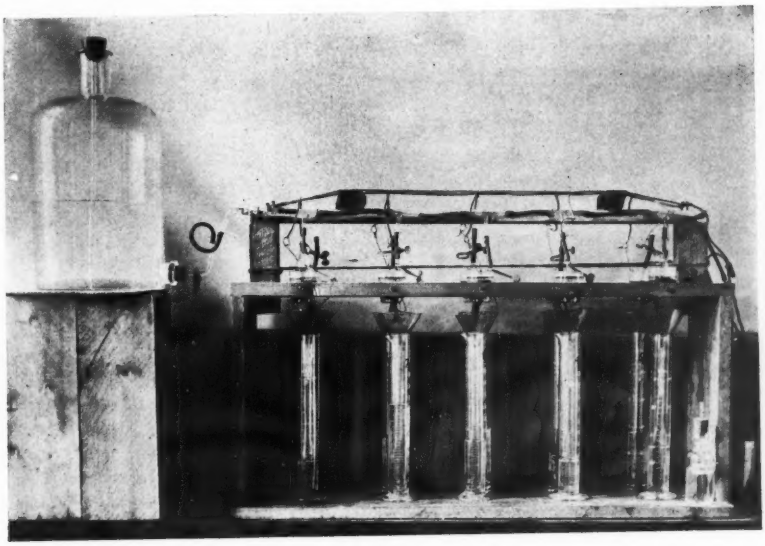
PLATE 1

TEN CELLS MOUNTED AND CONNECTED IN PARALLEL TO THE MAIN TERMINALS

SIMPLE ELECTRODIALYSIS CELL

M. L. M. SALGADO AND G. W. CHAPMAN

PLATE 1



THE INFLUENCE OF LIME ON THE RECOVERY OF TOTAL NITROGEN IN FIELD CROPS[1]

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Received for publication December 13, 1930

The amount of nitrogen removed by field crops has long been a subject of much interest to students of agricultural chemistry. Nearly 100 years ago Boussingault (1) began a series of experiments on his farm at Bechelbronn in Alsace, where he introduced quantitative methods in field studies. He weighed and analyzed the manures used and the crops obtained, and at the end of the rotation drew up a balance sheet for the purpose of studying gains and losses of nitrogen.

About 50 years later Lawes and Gilbert (3) made a careful study of the nitrogen removed by various crops in a number of different rotations. They summarized their results as follows:

> The average yield of nitrogen per acre per annum, was, with wheat, thirty-two years without manure, 20.7 pounds, and twenty-four years with a complex mineral manure, 22.1 pounds; with barley, twenty-four years without manure, 18.3 pounds, and twenty-four years with a complex mineral manure, 22.4 pounds; with root-crops, thirty-six years (including three of barley), with a complex mineral manure, 25.2 pounds; with beans, twenty-four years without manure, 31.3 pounds, and twenty-four years with a complex mineral manure, 45.5; with clover, six crops in twenty-two years, with one crop of wheat, three crops barley, and twelve years fallow, without manure, 30.5 pounds; with complex mineral manure, 39.8 pounds; with clover, on land which had not grown the crop for very many years, one year, 151.3 pounds; with a rotation of crops, seven courses, twenty-eight years, without manure, 36.8 pounds, and with superphosphate of lime, 45.2 pounds; with the mixed herbage of grass land, twenty years without manure, 33 pounds, and with complex mineral manure, including potash, 55.6; lastly, with Bokhara clover, five years, with mineral manure, between 80 and 90 pounds of nitrogen per acre per annum.
>
> The root-crops yielded more nitrogen than the cereal crops, and the leguminous crops very much more still.
>
> In all the cases of the experiments on ordinary arable land—whether with cereal crops, root-crops, leguminous crops, or a rotation of crops (excepting as yet the Bokhara clover)—the decline in the annual yield of nitrogen, none being supplied by manure, was very great.

Jenkins, Street, and Hubbell (2) found from nine series of tests with husking and silage corn at different experiment stations, that an acre of corn removed 87.5 pounds of nitrogen. In another experiment six tests gave for a 15-ton silage corn crop an average of 88 pounds of nitrogen an acre, and crops of husk-

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

ing corn at different places in Connecticut, calculated at 75 bushels an acre, gave an average of 92 pounds of nitrogen to the acre.

In the growing of continuous wheat with mineral fertilizers and legume green manure crops as a source of nitrogen, Lipman and Blair (5) found that wheat (including grain and straw) returned a 13-year average of 32.8 pounds of nitrogen an acre; with mineral fertilizers without nitrogen the return was 20.6 pounds of nitrogen an acre. Rye grown under the same conditions returned 34.9 pounds of nitrogen with mineral fertilizers and legume green manure, and 24.5 pounds with minerals only. In the annual report of the New Jersey station for 1923 (6), the same authors give figures to show that soybeans grown on well-limed soil returned through dry shelled beans and stalks, more than 100 pounds of nitrogen an acre. On unlimed land the average return for several varieties of beans was less than 15 pounds of nitrogen an acre.

In studying the problem of the removal of nitrogen by crops many factors must be considered. Among these may be mentioned climatic conditions, especially rainfall, the character of the soil, the soil reaction, the amount of fertilizer and lime used, cultural practices, etc.

In the work here reported an attempt is made to show the influence of two forms of lime on the nitrogen removed by the crops of some 5-year rotations.

EXPERIMENTAL

For a period of more than 20 years nitrogen determinations have been made on grain, hay, forage, and vegetable crops, grown on plots where calcium and magnesium limestone have been used in comparative tests. From these determinations and the dry weights, the amount of nitrogen removed through the crops, has been calculated.

The grain crops have included corn, oats, wheat, and barley; the hay has usually been timothy and clover; the forage crops have included soybeans, cowpeas, winter vetch, oats and peas, rye, millet, sorghum, and rape; the vegetable crops have been tomatoes, cucumbers, lima beans, carrots, and beets.

In the majority of cases the crop samples were first dried and nitrogen determinations made on the dry material. In some cases vegetables were weighed fresh and nitrogen determinations made on the fresh sample. The total nitrogen removed through the crop has been calculated on the acre basis.

The two forms of lime have been used in four different rotation systems:

Rotation 1—Corn, small grain, hay
Rotation 2—Corn, potatoes, grain, hay
Rotation 3—Corn, vegetable crops
Rotation 4—Corn, forage crops

The limestone is used in amounts equivalent to 1,000, 2,000, and 4,000 pounds an acre. Including one plot without lime, this gives seven plots for each system or 28 one-twentieth acre plots. The limestone has been applied at intervals of 5 years, preceding the corn. The no-lime plots have become noticeably

TABLE 1

Total nitrogen recovered in a 5-year rotation—1908–1912

LIMESTONE TREATMENT	1908	1909	1910	1911	1912	5-YEAR AVERAGE
			Rotation 1			
	Corn	Oats	Wheat	Oats*	Timothy and clover	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	38.5	17.6	27.5	30.1	16.3	26.0
½ ton Ca limestone	42.2	18.3	27.7	28.5	18.8	27.1
½ ton Mg limestone	56.1	24.8	38.5	40.0	25.7	37.0
1 ton Ca limestone	47.2	17.9	35.9	32.3	18.7	30.4
1 ton Mg limestone	51.5	27.4	38.7	38.9	28.4	37.0
2 tons Ca limestone	49.8	22.4	36.6	34.0	19.3	32.4
2 tons Mg limestone	52.4	29.6	38.9	43.0	29.8	38.7
			Rotation 2			
	Corn	Potatoes	Rye	Oats*	Timothy and clover	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	44.0	3.7	32.4	35.7	24.8	28.1
½ ton Ca limestone	63.3	3.6	40.6	41.2	33.5	36.4
½ ton Mg limestone	50.3	3.6	44.4	39.7	31.5	33.9
1 ton Ca limestone	Lost	3.3	38.2	38.2	30.4	27.5†
1 ton Mg limestone	42.6	3.2	49.1	41.8	30.7	33.5
2 tons Ca limestone	55.5	3.4	47.6	36.0	36.1	35.7
2 tons Mg limestone	47.7	3.4	55.0	37.8	30.2	34.8
			Rotation 3			
	Corn	Potatoes	Tomatoes	Lima beans	Cucumbers	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	41.5	2.4	24.2	19.5	5.8	18.7
½ ton Ca limestone	45.5	2.3	38.2	28.0	8.8	24.6
½ ton Mg limestone	42.7	2.9	34.2	26.3	8.3	22.9
1 ton Ca limestone	44.9	2.9	34.6	28.1	10.2	24.1
1 ton Mg limestone	49.8	3.4	34.3	27.7	5.8	24.2
2 tons Ca limestone	50.7	3.2	31.8	25.8	9.7	24.2
2 tons Mg limestone	53.5	2.8	39.0	24.6	5.0	25.0

TABLE 1—*Concluded*

LIMESTONE TREATMENT	1908	1909		1910		1911		1912		5-YEAR AVERAGE
	Rotation 4									
	Corn	Oats and peas	Millet	Rape	Vetch	Rye	Cow peas	Oats and peas	Cow peas	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	37.6	38.2	15.9	23.0	43.5	41.2	49.2	38.6	25.9	62.6
½ ton Ca limestone	39.8	40.3	16.8	30.8	59.9	43.8	62.3	58.2	32.0	76.8
½ ton Mg limestone	47.9	51.7	16.5	32.5	93.1	42.6	35.3	48.7	27.1	79.1
1 ton Ca limestone	42.0	32.0	18.7	36.3	93.5	51.0	65.6	58.8	44.2	88.4
1 ton Mg limestone	46.2	51.7	19.6	35.4	101.2	37.4	45.4	53.0	34.3	84.8
2 tons Ca limestone	53.9	40.2	19.2	44.2	93.0	47.8	50.7	42.2	41.9	86.6
2 tons Mg limestone	37.7	40.5	20.5	33.3	114.4	39.6	47.9	48.6	42.0	84.9

* Timothy and clover failed; oats substituted.

† Four-year average.

acid, whereas those that receive 1,000 and 2,000 pounds an acre are moderately to slightly acid, and those that receive 4,000 pounds are about neutral or slightly alkaline. The lime requirement of this soil was originally about 1,200 to 1,400 pounds CaO an acre. The soil of these plots lies so close to the older Penn soils, that it can hardly be considered a typical soil, but it is classed as Sassafras loam.

FERTILIZER TREATMENT

The fertilizer treatment has varied slightly, but in the majority of cases superphosphate has been used at the rate of about 300 pounds an acre, and muriate of potash at the rate of 50 to 100 pounds an acre. For non-legume crops a nitrogenous fertilizer has been used to give the equivalent of about 150 to 200 pounds of nitrate of soda an acre. Only small amounts of nitrogen have been used for the legumes in the forage crops rotation. No farm manure has been used. Results for the four 5-year periods, for the four rotations are shown in tables 1 to 4.

Legume crops grown on the limed plots have almost invariably shown a higher percentage of nitrogen than those grown on the unlimed plots. In the majority of cases also they have shown a higher yield of dry matter. Thus with increase in percentage of nitrogen and also in yield, there has been a decided increase in the amount of total nitrogen returned per acre. In a number of cases also non-legume crops grown on limed plots have shown a somewhat higher percentage of nitrogen than those grown on the unlimed plot.

In the forage crops rotation two crops were grown during 4 of the 5 years,

TABLE 2

Total nitrogen recovered in a 5-year rotation—1913–1917

LIMESTONE TREATMENT	1913	1914	1915	1916		1917	5-YEAR AVERAGE
Rotation 1							
	Corn	Oats	Wheat	Timothy and clover*		Timothy and clover*	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	25.6	19.7	24.1	31.2	4.3	19.9	25.0
½ ton Ca limestone	33.1	17.0	28.3	41.3	25.0	28.5	34.6
½ ton Mg limestone	41.2	21.5	30.5	45.8	28.9	27.8	39.1
1 ton Ca limestone	40.3	22.2	31.5	52.7	35.9	30.9	42.7
1 ton Mg limestone	52.3	23.4	29.6	54.8	51.3	32.3	48.7
2 tons Ca limestone	49.3	20.8	28.7	52.6	45.0	29.7	45.2
2 tons Mg limestone	54.2	24.0	29.7	52.2	47.6	34.6	48.5
Rotation 2							
	Corn	Potatoes	Rye	Timothy and clover*		Timothy and clover*	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	33.3	22.5	28.2	39.0	14.9	27.3	33.0
½ ton Ca limestone	50.6	23.8	26.7	38.6	23.0	29.4	38.4
½ ton Mg limestone	56.5	21.7	29.4	52.7	26.5	34.4	44.2
1 ton Ca limestone	61.2	20.9	29.5	56.3	26.5	39.0	46.7
1 ton Mg limestone	62.1	21.1	30.6	46.2	44.6	37.5	48.4
2 tons Ca limestone	70.4	20.3	28.8	62.2	39.6	36.0	51.5
2 tons Mg limestone	60.9	19.3	28.5	55.2	41.5	34.7	48.0
Rotation 3							
	Corn	Potatoes	Tomatoes	Lima beans		Cucumbers	
	lbs.	*lbs.*	*lbs.*	*lbs.*		*lbs.*	*lbs.*
No limestone	43.0	15.4	40.6	16.7		9.5	25.0
½ ton Ca limestone	49.1	16.2	48.1	22.4		14.8	30.1
½ ton Mg limestone	46.5	16.4	42.9	19.3		16.9	28.4
1 ton Ca limestone	53.4	19.1	46.9	29.4		18.3	33.4
1 ton Mg limestone	56.3	19.3	46.7	23.2		16.9	32.5
2 tons Ca limestone	55.4	20.7	43.1	22.8		15.0	31.4
2 tons Mg limestone	62.1	16.3	48.7	14.3		15.9	31.5

TABLE 2—*Concluded*

LIMESTONE TREATMENT	1913	1914		1915		1916		1917		5-YEAR AVERAGE
Rotation 4										
	Corn	Oats and peas	Millet	Vetch	Rape	Rye	Cow peas	Oats and peas	Cow peas	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	37.0	25.2	4.7	7.1	16.7	23.5	28.5	27.4	32.3	40.5
½ ton Ca limestone	45.3	41.2	6.3	28.7	18.4	29.5	28.3	42.6	34.8	55.0
½ ton Mg limestone	39.8	37.9	2.2	60.1	19.2	30.8	54.9	57.6	47.2	69.9
1 ton Ca limestone	52.1	41.6	8.8	49.6	22.1	28.4	41.1	49.0	46.7	67.9
1 ton Mg limestone	43.0	35.7	3.8	86.5	23.3	30.5	65.4	54.6	50.4	78.6
2 tons Ca limestone	55.4	46.8	7.3	65.8	24.4	36.1	59.7	56.1	64.9	83.3
2 tons Mg limestone	44.8	38.8	4.1	89.6	27.1	23.1	54.9	64.1	55.3	80.4

* Two cuttings of timothy and clover.

with the exception of 1924. This makes 35 crops on these plots for the 20 years. In this connection it may be pointed out that the crops in this rotation have returned much more nitrogen than those of the other rotations. This is, of course, mainly because most of these crops were legumes and therefore got a part of their nitrogen from the air. It will be remembered that very little nitrogen has been applied for these crops. This is of special interest because it shows that where lime and minerals are used with care, yields may be kept up to a fair level over a period of years without the use of farm manure and with a minimum of nitrogenous fertilizers. With the exception of the check plot the average nitrogen returns for this rotation for the last 5 years are about as high as for the first 5 years.

FIRST 5-YEAR PERIOD

The figures for the first 5-year period are shown in table 1. Reference to the averages shows that excepting the check plot, rotations 1 and 2 returned about 30 to 35 pounds nitrogen to the acre. Rotation 3 returned about 24 pounds, and rotation 4 a little better than 80 pounds.

In nearly all cases the no-lime plots gave a lower nitrogen return than the limed plots, though in the case of potatoes and vegetables the difference is slight. In many cases the 1,000- and 2,000-pound applications have given about as high a nitrogen yield as the 4,000-pound application. The failure of the timothy and clover in rotations 1 and 2 in 1911 undoubtedly reduced the nitrogen yield for that year.

With the exception of rotation 1, the 5-year average indicates that the two forms of limestone had about the same effect with reference to the amount of

TABLE 3

Total nitrogen recovered in a 5-year rotation—1918–1922

LIMESTONE TREATMENT	1918	1919	1920	1921	1922		5-YEAR AVERAGE
				Rotation 1			
	Corn	Oats	Barley*	Timothy hay†	Timothy hay†		
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	48.3	24.4	12.9	14.9	15.1	1.5	23.4
½ ton Ca limestone	54.6	34.4	25.3	22.3	30.2	2.5	33.9
½ ton Mg limestone	60.3	35.7	28.6	26.2	46.5	9.8	41.4
1 ton Ca limestone	55.8	35.8	26.1	23.9	52.7	7.7	40.4
1 ton Mg limestone	62.6	42.1	31.2	30.6	48.1	12.6	45.4
2 tons Ca limestone	57.7	36.0	28.9	24.5	61.8	14.7	44.7
2 tons Mg limestone	64.8	41.5	31.2	28.4	63.0	23.6	50.5
				Rotation 2			
	Corn	Potatoes	Rye	Timothy hay†		Timothy hay†		
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	55.0	16.9	27.4	18.7	6.1	32.9	1.9	31.8
½ ton Ca limestone	52.3	23.8	29.3	20.3	11.0	51.2	13.3	40.2
½ ton Mg limestone	57.1	27.9	32.9	20.3	11.1	45.0	17.7	42.4
1 ton Ca limestone	63.7	26.8	31.5	21.2	23.3	67.4	20.3	50.8
1 ton Mg limestone	60.0	20.3	34.5	21.7	26.1	66.5	30.3	51.9
2 tons Ca limestone	67.3	23.3	37.2	18.0	17.3	58.4	21.7	48.6
2 tons Mg limestone	69.2	25.2	43.0	19.4	31.8	44.0	28.8	52.3
				Rotation 3			
	Corn	Potatoes	Tomatoes	Lima beans	Cucumbers		
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*		*lbs.*
No limestone	58.5	23.3	35.0	12.6	5.5		27.0
½ ton Ca limestone	68.7	29.0	28.9	19.8	9.4		31.2
½ ton Mg limestone	69.9	19.4	31.8	36.6	8.9		33.3
1 ton Ca limestone	72.0	26.8	26.3	31.9	10.4		33.5
1 ton Mg limestone	70.1	26.9	34.0	43.3	9.7		36.8
2 tons Ca limestone	72.0	22.7	19.9	49.2	13.1		35.4
2 tons Mg limestone	72.9	30.7	29.8	37.0	8.6		35.8

TABLE 3—*Concluded*

LIMESTONE TREATMENT	1918	1919		1920		1921		1922		5-YEAR AVERAGE
Rotation 4										
	Corn	Oats and peas	Millet	Rye and vetch	Rape	Rye and vetch	Soy-beans	Rye	Soy-beans	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	59.4	27.2	9.3	17.8	15.1	33.7	27.3	19.0	31.9	48.1
½ ton Ca limestone	61.6	49.3	13.2	31.7	13.9	79.0	41.1	20.6	61.4	74.4
½ ton Mg limestone	62.8	44.3	10.8	40.8	7.0	92.1	64.5	28.2	59.4	82.0
1 ton Ca limestone	56.6	54.1	16.6	28.0	13.1	102.5	73.3	17.8	66.8	85.6
1 ton Mg limestone	58.3	43.3	11.4	62.5	11.7	115.1	75.9	32.3	82.9	98.7
2 tons Ca limestone	66.7	55.1	16.9	40.1	15.0	95.4	68.3	34.9	85.8	95.6
2 tons Mg limestone	56.5	44.6	12.6	54.3	15.3	101.9	70.6	21.2	96.1	94.6

* Wheat winter-killed; barley substituted.

† Clover seeded with timothy was largely a failure.

nitrogen returned in the crop. There are, it is true, many individual variations, but in the majority of cases the difference is not great.

SECOND 5-YEAR PERIOD

The total yields of nitrogen for the second 5-year period are shown in table 2. They do not differ greatly from those of the first 5 years. There were two cuttings of timothy and clover in 1916, and this gave a decided increase in nitrogen for rotations 1 and 2. The limed plots of these two rotations returned a 5-year average of about 45 pounds nitrogen to the acre. The 5-year average for the limed plots of rotation 3 is close to 30 pounds an acre, and the corresponding plots of the forage crop rotation gave about 72 pounds an acre.

THIRD 5-YEAR PERIOD

The results for the third 5-year period are shown in table 3. The general averages are quite close to the averages for the second 5-year period. The returns from the corn crop are, however, distinctly higher than for either the first or second 5-year periods. The 5-year average for the limed plots of rotation 1 is a little better than 40 pounds an acre; for rotation 2 it stands between 45 and 50; for rotation 3 it is close to 35, and for rotation 4 nearly 90 pounds an acre. This latter is equivalent in amount to over 500 pounds nitrate of soda an acre. In a majority of cases the 4,000-pound application of limestone gave the highest nitrogen returns, but it frequently happens that the increase with this amount over the yield with 2,000 pounds is only slight. The averages for this period indicate that the yields were slightly higher with the magnesian

TABLE 4

Total nitrogen recovered in a 5-year rotation—1923–1927

LIMESTONE TREATMENT	1923	1924	1925	1926		1927	5-YEAR AVERAGE
Rotation 1							
	Corn	Oats	Wheat	Timothy and clover*		Timothy	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	37.5	23.7	34.1	12.5	....	20.8	25.7
½ ton Ca limestone	56.7	35.7	45.6	20.0	6.0	35.7	39.9
½ ton Mg limestone	66.7	36.7	50.0	22.9	26.0	50.3	50.5
1 ton Ca limestone	62.5	33.4	46.0	21.7	31.3	54.0	49.8
1 ton Mg limestone	70.1	36.9	45.0	28.3	36.9	52.4	53.9
2 tons Ca limestone	67.9	32.6	51.9	19.5	27.3	59.8	51.8
2 tons Mg limestone	71.2	36.5	38.8	28.9	41.7	62.7	56.0
Rotation 2							
	Corn	Corn	Oats	Timothy and clover*		Timothy	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	47.4	50.2	26.9	19.7	2.1	25.7	34.4
½ ton Ca limestone	64.3	57.7	27.6	20.5	6.7	45.8	44.5
½ ton Mg limestone	64.3	59.4	29.2	21.3	18.1	59.5	50.4
1 ton Ca limestone	72.6	58.3	28.2	23.0	32.4	55.4	54.0
1 ton Mg limestone	67.7	66.2	29.5	24.4	27.5	60.9	55.2
2 tons Ca limestone	76.8	68.3	29.5	23.9	35.4	70.5	60.9
2 tons Mg limestone	73.5	70.0	30.1	27.5	30.8	48.7	56.1
Rotation 3							
	Corn	Corn	Rape	Sorghum		Beets	
	lbs.	*lbs.*	*lbs.*	*lbs.*		*lbs.*	*lbs.*
No limestone	63.3	53.8	42.9	29.6		....	37.9
½ ton Ca limestone	67.9	58.2	58.3	68.4		18.0	54.2
½ ton Mg limestone	66.9	50.3	54.1	105.8		22.3	59.9
1 ton Ca limestone	75.0	54.3	67.1	67.0		41.2	60.9
1 ton Mg limestone	73.7	52.0	67.4	71.7		31.3	59.2
2 tons Ca limestone	78.1	58.9	72.2	71.3		43.4	64.8
2 tons Mg limestone	70.5	57.8	83.5	70.5		34.3	63.3

TABLE 4—*Concluded*

LIMESTONE TREATMENT	1923	1924	1925		1926		1927		5-YEAR AVERAGE
Rotation 4									
	Corn	Oats and peas	Rye and vetch	Soy-beans	Rye and vetch	Soy-beans	Oats and peas	Soy-beans	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
No limestone	39.7	25.0	32.4	26.0	19.9	21.5	28.6	25.7	43.8
½ ton Ca limestone	56.1	42.3	36.4	51.8	22.5	47.4	36.1	48.0	68.1
½ ton Mg limestone	64.5	35.5	35.8	62.6	23.6	63.9	30.2	50.5	73.3
1 ton Ca limestone	60.7	39.2	36.6	63.9	20.4	81.1	42.9	55.7	80.1
1 ton Mg limestone	55.4	36.2	37.0	88.8	20.2	92.8	37.0	73.5	88.2
2 tons Ca limestone	67.6	35.5	45.7	84.5	27.5	86.2	43.9	89.4	96.1
2 tons Mg limestone	59.4	33.9	35.1	77.7	24.5	101.4	40.1	59.7	86.4

* Timothy and clover, two cuttings.

than with the calcium limestone. In the forage crop rotation the 4,000-pound application of limestone gave a nitrogen return of approximately 95 pounds an acre. Vegetables and potatoes usually give a rather low yield of nitrogen. The timothy and clover crop of 1921 was small and therefore gave a low yield of nitrogen.

FOURTH 5-YEAR PERIOD

The figures for the nitrogen returned through the crop during the fourth 5-year period are shown in table 4. It is interesting to note that the yields for rotations 1, 2, and 3 for this period are higher than for any of the three preceding periods. This is a fair indication that the nitrogen supply of the soil is being maintained, otherwise the yields would show a decrease rather than an increase. Here it may be pointed out that the vegetable rotation was changed for this period, only one vegetable crop—beets—being included.

The yields for the forage crop rotation have been well maintained, the general average for the limed plots being 82 pounds of nitrogen to the acre. In this rotation there is little difference between the nitrogen recovered with the two forms of limestone. Generally the returns with 4,000 pounds of the limestone are more than with either the 2,000 or 1,000 pounds.

YIELD OF DRY MATTER

The yields of dry matter (in some cases field weights of fresh vegetables) for the last 10 years are reported in tables 5 and 6, so that they may be studied in connection with the nitrogen data. The corresponding figures for the first 10 years have appeared in earlier publications (4, 7, 8).

TABLE 5

Yields of dry matter in a 5-year rotation—1918–1922

LIMESTONE TREATMENT	CORN—1918		OATS—1919		BARLEY—1920		TIMOTHY HAY*—1921		TIMOTHY HAY*—1922		5-YEAR AVERAGE
	Grain	Stover	Grain	Straw	Grain	Straw	First crop	Second crop	First crop	Second crop	
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Rotation 1											
None	2,328	1,820	720	1,600	452	588	1,700		1,600	136	2,189
½ ton Ca limestone	2,508	1,858	680	2,440	1,124	1,236	2,860		3,680	144	3,306
½ ton Mg limestone	2,716	2,056	720	2,400	1,144	1,476	3,420		4,040	660	3,726
1 ton Ca limestone	2,752	1,958	560	2,520	1,080	1,340	2,760		4,520	464	3,591
1 ton Mg limestone	2,620	2,314	680	2,720	1,216	1,584	3,680		4,800	810	4,085
2 tons Ca limestone	2,632	2,052	720	2,560	1,196	1,384	2,840		4,480	870	3,747
2 tons Mg limestone	2,988	2,208	640	2,720	1,200	1,420	3,560		4,560	1,480	4,155
Rotation 2											
			POTATOES†								4-YEAR AVERAGE
			Primes	Seconds							
			bu.	*bu.*							
None	2,580	1,868	80.0	10.2	1,096	2,824	2,830	34	3,480	120	3,708
½ ton Ca limestone	2,468	2,140	81.1	12.5	1,280	3,520	3,220	128	4,560	800	4,529
½ ton Mg limestone	2,620	2,242	89.8	16.5	1,356	3,404	2,640	240	4,080	1,100	4,421
1 ton Ca limestone	2,972	2,310	97.8	13.8	1,212	3,868	2,970	600	5,440	1,360	5,183
1 ton Mg limestone	2,660	2,140	97.4	13.2	1,392	3,608	2,630	840	4,160	1,920	4,838
2 tons Ca limestone	3,032	2,238	82.8	13.2	1,440	4,240	2,320	520	4,360	1,320	4,868
2 tons Mg limestone	2,944	2,212	84.5	15.8	1,644	4,396	2,400	1,100	3,920	1,800	5,104
Rotation 3											
					TOMATOES†	LIMA BEANS, DRY	CUCUMBERS†				
					lbs.	*lbs.*	*lbs.*				
None	2,816	1,968	101.1	18.3	18,728	380	5,218	.....			
½ ton Ca limestone	2,904	2,374	147.8	24.1	15,034	620	8,948	.....			
½ ton Mg limestone	2,736	2,200	69.7	20.8	16,664	1,050	9,228	.....			
1 ton Ca limestone	3,064	2,468	137.8	22.1	13,768	950	9,496	.....			
1 ton Mg limestone	2,944	2,362	165.8	25.0	17,747	1,330	9,110	.....			
2 tons Ca limestone	3,108	2,286	106.0	18.5	9,899	1,370	12,726	.....			
2 tons Mg limestone	3,100	2,286	146.4	26.2	16,352	950	8,408	.....			

TABLE 5—*Concluded*

LIMESTONE TREATMENT	CORN—1918		OATS AND PEA HAY	MILLET	RYE AND VETCH	RAPE	RYE AND VETCH	SOY-BEAN HAY	RYE	SOY-BEAN HAY	4-YEAR AVERAGE
	Grain	Stover									
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Rotation 4											
None	2,600	1,968	1,960	800	2,360	1,840	2,950	2,120	1,800	2,120	4,104
½ ton Ca limestone	2,752	2,222	3,640	1,040	2,840	1,168	4,660	2,900	2,800	3,120	5,428
½ ton Mg limestone	2,668	2,166	3,240	800	3,080	520	5,180	3,700	3,200	3,080	5,527
1 ton Ca limestone	2,760	2,134	3,640	1,160	2,200	1,172	4,988	3,220	3,120	2,960	5,471
1 ton Mg limestone	2,620	1,868	3,200	800	3,800	1,020	5,080	4,520	3,240	3,240	5,878
2 tons Ca limestone	2,964	2,166	3,920	1,000	3,440	1,120	5,292	3,880	4,120	3,160	6,212
2 tons Mg limestone	2,488	2,066	3,000	920	3,440	1,140	5,000	3,620	3,160	3,240	5,615

* Clover seeded with timothy was largely a failure.

† Field weights—not included in averages.

Table 5, which gives results for the 5 years 1918–1922, shows average annual yields for the limestone treated plots, varying from about 3,500 to 6,000 pounds total dry matter an acre. The largest total yields are found in rotation 4 where in 4 out of the 5 years two crops were grown each year.

Generally, the averages show an increase for the limed plots over the plots without lime amounting to more than 1,000 pounds an acre. In some cases this increase amounts to 2,000 pounds or more.

The results with the two forms of limestone do not always agree closely, but the differences are not consistently in favor of either one.

The yields for the 5 years 1923–1927 are shown in table 6. With the exception of rotation 1, the averages are slightly lower than for the preceding 5 years. Here also as in the preceding 5 years the limed plots show a decided increase over the plots without lime. Also the yields with the two forms of lime are generally in fair agreement.

THE PERCENTAGE OF NITROGEN IN DRY MATTER

Table 7 shows the percentage of nitrogen in the crops of the four rotations for the period 1923–1927 inclusive. These figures are of interest as showing the amount of nitrogen removed by 100 pounds of dry matter in the various crops.

It will be noted that the grains and soybean hay are comparatively high in nitrogen; corn stover contains approximately 0.6 to 0.9 per cent nitrogen; oats straw contains almost as much as the stover, whereas wheat straw is low in nitrogen. The low percentage of nitrogen in the timothy and clover (first cutting) indicates that there was very little clover with the timothy. A nitro-

TABLE 6

Yields of dry matter in a 5-year rotation—1923–1927

LIMESTONE TREATMENT	CORN—1923		OATS—1924		WHEAT—1925		TIMOTHY AND CLOVER—1926		TIMOTHY HAY—1927	5-YEAR AVERAGE
	Grain	Stover*	Grain	Straw	Grain	Straw	First crop	Second crop		
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Rotation 1										
None	1,740	2,188	671	1,346	1,053	2,644	1,242	.....	1,887	2,554
½ ton Ca limestone	2,483	2,406	1,108	1,950	1,545	4,306	2,476	230	3,789	4,059
½ ton Mg limestone	2,860	3,270	1,223	2,170	1,671	4,601	2,578	1,058	5,059	4,898
1 ton Ca limestone	2,973	3,010	1,041	2,038	1,642	4,421	2,370	1,210	5,320	4,805
1 ton Mg limestone	2,959	2,855	1,190	2,194	1,566	4,421	2,871	1,393	5,165	4,923
2 tons Ca limestone	2,920	3,031	1,037	2,108	1,668	4,777	2,039	1,072	5,433	4,817
2 tons Mg limestone	2,903	2,829	1,134	2,101	1,400	3,863	2,801	1,654	5,447	4,826
Rotation 2										
			CORN		OAT HAY					
None	2,315	2,765	2,463	2,110	1,620		2,550	80	3,253	3,431
½ ton Ca limestone	2,793	2,884	2,700	2,825	1,860		2,561	264	4,424	4,062
½ ton Mg limestone	2,828	2,904	2,813	3,180	1,800		2,846	603	5,094	4,414
1 ton Ca limestone	2,903	3,108	2,650	2,885	1,760		2,469	1,173	5,137	4,417
1 ton Mg limestone	2,643	3,017	3,013	3,000	1,860		2,585	965	5,701	4,557
2 tons Ca limestone	2,863	3,855	2,788	2,770	1,880		2,656	1,326	5,278	4,683
2 tons Mg limestone	2,835	3,341	3,050	2,995	1,880		2,705	1,048	4,396	4,450
Rotation 3										
					RAPE	SORGHUM	CARROTS**			4-YEAR AVERAGE
None	2,213	3,284	2,538	2,535	1,700		2,209		300	3,620
½ ton Ca limestone	2,660	2,606	2,638	2,805	2,400		5,109		2,440	4,555
½ ton Mg limestone	2,425	3,407	2,363	2,800	2,350		7,211		5,320	5,139
1 ton Ca limestone	2,803	3,775	2,550	2,915	2,800		4,883		8,960	4,932
1 ton Mg limestone	2,870	3,330	2,725	2,495	2,850		4,996		8,600	4,817
2 tons Ca limestone	2,675	3,971	2,750	2,735	2,900		5,073		10,720	5,026
2 tons Mg limestone	2,730	2,913	2,575	2,715	3,050		5,483		10,440	4,867

TABLE 6—*Concluded*

Limestone treatment	CORN—1923		OATS AND PEA HAY	RYE AND VETCH†	SOY-BEAN HAY	RYE AND VETCH	SOY-BEAN HAY	OATS AND PEA HAY	SOY-BEAN HAY	5-YEAR AVERAGE
	Grain	Stover*								
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Rotation 4										
None	1,628	1,764	1,757	3,800	1,220	2,374	1,682	2,466	1,294	3,597
½ ton Ca limestone	2,320	2,396	2,584	4,040	2,220	2,723	2,303	2,985	2,116	4,737
½ ton Mg limestone	2,745	2,998	2,570	4,040	2,560	2,723	2,710	3,083	2,166	5,119
1 ton Ca limestone	2,460	2,628	2,476	3,880	2,720	2,370	2,992	3,542	1,996	5,013
1 ton Mg limestone	2,713	3,095	2,552	4,240	3,160	2,159	2,971	3,267	2,367	5,305
2 tons Ca limestone	3,213	2,818	2,387	5,000	3,200	3,086	2,985	3,775	2,533	5,799
2 tons Mg limestone	2,730	3,217	2,376	3,920	2,960	2,356	3,189	3,436	2,035	5,244

* Cobs not included.

† Mostly rye.

** Field weights—omitted from average.

gen percentage above two and one-half for the second cutting indicates clover as the predominant crop. The rye and vetch show a low percentage of nitrogen because there was very little vetch with the rye.

SUMMARY

Over a period of 20 years nitrogen determinations have been made on a variety of field crops grown on four separate 5-year rotations, with varying amounts of calcium and magnesian limestone.

The total nitrogen thus recovered has been calculated and reported for four 5-year periods.

The highest returns have been from the forage crops rotation where two crops a year—mostly legumes—have been grown 3 or 4 years out of the 5. Generally the corn crops have given the next highest returns and the vegetable and potato crops the lowest.

The yields of timothy and clover were low in 1912, 1917, and 1921, and consequently this influenced the yield of total nitrogen.

In most cases the yield of nitrogen was almost as high with 2,000 pounds of the limestone as with 4,000 pounds.

Taking the 5-year averages for the different rotations, the yields of nitrogen are very nearly the same with the calcium and magnesian limestone, though in some cases the latter gave slightly higher yields.

For the most part the yields of nitrogen are higher for the fourth 5-year period than for the earlier periods. This would indicate that the general

TABLE 7

Nitrogen in the crops of a 5-year rotation—1923–1927

LIMESTONE TREATMENT	CORN—1923		OATS—1924		WHEAT—1925		TIMOTHY AND CLOVER—1926		TIMOTHY HAY—1927
	Grain	Stover	Grain	Straw	Grain	Straw	First crop	Second crop	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Rotation 1									
None	1.29	0.641	1.98	0.774	2.33	0.360	1.00	.....	1.10
½ ton Ca limestone	1.47	0.789	2.14	0.616	2.17	0.281	0.81	2.60	0.94
½ ton Mg limestone	1.46	0.720	2.12	0.493	2.14	0.309	0.89	2.46	1.01
1 ton Ca limestone	1.44	0.609	2.18	0.528	2.08	0.267	0.91	2.59	1.02
1 ton Mg limestone	1.52	0.830	2.13	0.528	2.08	0.281	0.99	2.65	1.02
2 tons Ca limestone	1.52	0.726	2.11	0.510	2.27	0.295	0.96	2.55	1.10
2 tons Mg limestone	1.55	0.868	2.11	0.598	2.01	0.276	1.03	2.52	1.15

			CORN		OAT HAY			
			Grain	Stover				
Rotation 2								
None	1.39	0.521	1.35	0.756	1.66	0.77	2.57	0.79
½ ton Ca limestone	1.56	0.656	1.40	0.654	1.48	0.80	2.55	1.04
½ ton Mg limestone	1.47	0.742	1.34	0.647	1.62	0.75	3.00	1.17
1 ton Ca limestone	1.60	0.783	1.30	0.782	1.60	0.93	2.76	1.08
1 ton Mg limestone	1.55	0.846	1.37	0.774	1.59	0.95	2.85	1.07
2 tons Ca limestone	1.58	0.789	1.53	0.891	1.57	0.90	2.67	1.34
2 tons Mg limestone	1.54	0.852	1.55	0.709	1.60	1.02	2.94	1.11

					RAPE	SORGHUM	BEETS*	BEET TOPS*
Rotation 3								
None	1.55	0.852	1.33	0.738	2.53	1.34	.....	.....
½ ton Ca limestone	1.57	0.956	1.38	0.727	2.43	1.34	0.208	0.302
½ ton Mg limestone	1.58	0.805	1.31	0.654	2.30	1.47	0.233	0.376
1 ton Ca limestone	1.57	0.783	1.41	0.582	2.40	1.37	0.202	0.327
1 ton Mg limestone	1.50	0.877	1.34	0.582	2.36	1.44	0.210	0.330
2 tons Ca limestone	1.58	0.868	1.33	0.764	2.49	1.41	0.196	0.354
2 tons Mg limestone	1.57	0.909	1.36	0.793	2.74	1.29	0.220	0.301

TABLE 7—*Concluded*

Limestone treatment	CORN—1923		OATS AND PEAS	RYE AND VETCH†	SOY-BEANS	RYE AND VETCH	SOY-BEANS	OATS AND PEAS	SOY-BEANS
	Grain	Stover							
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
				Rotation 4					
None	1.52	0.805	1.43	0.852	2.13	0.84	1.28	1.16	1.98
½ ton Ca limestone	1.45	0.884	1.64	0.900	2.33	0.83	2.06	1.21	2.27
½ ton Mg limestone	1.47	0.773	1.38	0.886	2.45	0.87	2.36	0.98	2.33
1 ton Ca limestone	1.49	0.868	1.58	0.942	2.35	0.86	2.71	1.21	2.79
1 ton Mg limestone	1.36	0.562	1.42	0.872	2.81	0.94	3.12	1.13	3.10
2 tons Ca limestone	1.41	0.742	1.49	0.914	2.64	0.89	2.89	1.16	3.53
2 tons Mg limestone	1.37	0.641	1.43	0.895	2.62	1.04	3.18	1.17	2.93

* Fresh materials not dried.

† Very little vetch in this crop.

fertility level of the soil is being maintained, and perhaps slightly raised, under the systems of cropping employed.

In nearly all cases unlimed plots returned less nitrogen and a lower crop yield than the limed plots.

In the case of legume crops the limed plots almost invariably yielded dry matter with a higher percentage of nitrogen than the unlimed plots.

Tables showing the yields of dry matter for the four rotations for the 10-year period 1918–1927, are included; also a table giving percentage of nitrogen in the dry matter of the different crops for the 5-year period 1923–1927.

Attention is called to the fact that no farm manure has been used and that the amount of commercial fertilizer applied has been moderate. Note is made also of the fact that the yields, as indicated by the total nitrogen returned, have increased slightly.

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DOUBLE INFECTION OF LEGUMINOUS PLANTS WITH GOOD AND POOR STRAINS OF RHIZOBIA[1]

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Received for publication December 17, 1930

The demonstration that strains of Rhizobia vary in their abilityto aid the growth of the leguminous plant raises many questions of practical importance. Certain strains of the pea nodule bacteria (5) and of the clover nodule organism (1) have been shown to be essentially parasitic. These ineffective or poor strains[2] seem to be widely distributed in nature; their presence in a field may be responsible for low yields or even crop failure, according to Leonard (9). The early studies relating to this problem are summarized by Hiltner (6). Knowledge of the relative infective power of the good and poor strains of the nodule organism and of the reaction of the host plant upon infection with these types is essential to any attempt to evaluate the losses due to such parasitic strains and to any program looking toward the replacement of the poor strains in the field with better ones. The investigations reported here give an answer to some of these questions.

ISOLATION STUDIES ON PLANTS INOCULATED WITH BOTH GOOD AND POOR STRAINS

A number of investigators have reported the simultaneous presence of two or more strains of the nodule bacteria on a single host plant. All of these reports, however, have been based either upon cultural, morphological, or serological differences in the strains rather than on their efficiency in aiding plant growth.

Greig-Smith (4), in 1906, reported isolating culturally distinct strains of Rhizobia from a single nodule of lupine; and in 1907 de Rossi reported the presence of two types of the nodule organism in plates made from a single nodule (11). In neither case is adequate proof given that the organisms found were actually the nodule organism. Beijerinck (2) stated that with lupine and

[1] Published with the approval of the director.

[2] Definitions of certain terms as used in this paper are as follows:

Inoculation—the act of applying the Rhizobium to seed, soil, or plants.

Infection—the entrance of the Rhizobium into the plant with the production of nodules on the plants.

Effective or good strain—a strain of Rhizobium capable of infecting and of aiding the growth of a particular host plant when it is grown in a nitrogen-deficient medium.

Ineffective or poor strain—a strain of Rhizobium capable of infecting but giving little or no aid to the growth of a particular host plant when it is grown in a nitrogen-deficient medium.

serradella there are different kinds of bacteria isolated from the nodules at different seasons of the year. His method of distinguishing between the types is not explained.

Serological investigations have offered a definite method ofdistinguishing between strains of the same species and have been used by several workers. Bialosuknia and Klott (3) found two strains belonging to different serological groups on a single plant of broad bean. Okawara and Yoshida (10) reported the same phenomenon with peas and vetch. A similar report, was made by Jimbo (8) working with Robinia. Israilsky (7), on the contrary, failed to find more than one type of nodule organism on a single plant, and stated that, after being infected by one strain of the organism, the plant is not susceptible to infection by another type. This agrees with the earlier views of Hiltner (6), who believed that only strains of higher virulence could invade plants already bearing nodules.

W. B. Sarles, of this station, in some preliminary experiments on this problem found it possible to cause simultaneous infection of soybeans with two serologically different strains of *Rhizobium japonicum* and in one instance found two strains in a single nodule.

A personal communication from Gray at the Rothamsted Experimental Station states that two different strains of the nodule organism were isolated from a single nodule of alfalfa. Coalescence of two nodules which started their growth from closely adjacent root hairs may occur. The presence of two different strains of nodule bacteria in such a nodule might be expected.

Technic

To test the possibility of a single plant's bearing nodules formed by both effective and ineffective strains, plants of alfalfa, clover, peas, and soybeans were grown under carefully controlled conditions and inoculated with representative effective and ineffective strains. Later, isolations were made from a number of the nodules to recover the strains. With each host plant the following series were carried:

I. Uninoculated
II. Inoculated at planting with the poor strain
III. Inoculated at planting with the good strain
IV. Inoculated at planting with both strains
V. Inoculated with the poor strain at planting and 4 to 6 weeks later inoculated with the good strain.
VI. Inoculated with the good strain at planting and 4 to 6 weeks later inoculated with the poor strain.

In series V and VI, at the time of the second inoculation, the pots were allowed to dry almost to the wilting point of the plants, and a heavy inoculation was made on the surface of the sand and immediately washed in with sterile water.

The seeds were treated with mercuric chloride to render them bacteria-free and were then planted in a sterilized glacial sand, low in nitrogen and of an

alkaline reaction. The plants were watered with sterile distilled water. Mineral nutrients were supplied at intervals using a modified Crone's solution.[3] The soybeans and peas were grown in open half-gallon pots, and the clover and alfalfa in cotton-stoppered glass bottles with duplicate sets in open half-gallon pots. All plants were grown in a greenhouse reserved for this work, and every precaution was taken to avoid contamination with other strains of Rhizobia.

TABLE 1

History and description of the cultures used

CULTURE NUMBER	HOST ADAPTATION	DATE OBTAINED	GROWTH ON MANNITOL YEAST-WATER AGAR	FERMENTATION CHARACTERS	LIMITING pH IN A PEPTONE, SUCROSE SOLUTION
			Rhizobium meliloti		
100	Good	1912	Raised growth, considerable slime	Acid forming*	
101	Poor	1915	Thin growth, little slime	Non-acid forming	
			Rhizobium trifolii		
202	Poor	1921	Medium slime, unevenly opaque	Acid forming	
205	Good	1922	Considerable slime, evenly opaque	Non-acid forming	
			Rhizobium leguminosarum		
310	Poor	1924	Unevenly opaque	Non-acid forming	
313	Good	1924	Evenly opaque	Acid forming	
			Rhizobium japonicum		
501	Good	1918	Moderate growth		4.4
502	Poor	1921	Thin growth		5.0

* The terms *acid forming* and *non-acid forming* are relative only. The *Rhiz. meliloti* were tested against lactose, glycerol, and mannitol; *Rhiz. trifolii* against glycerol and mannitol and *Rhiz. leguminosarum* against mannitol.

Two strains each of *Rhizobium meliloti, Rhiz. trifolii, Rhiz. leguminosarum Rhiz. japonicum,* which were tested on alfalfa, red clover, garden pea, and soybean respectively, were selected to give as great a contrast as possible in their effect on the host plant and in their cultural characteristics. A description of these cultures is given in table 1. In addition to the cultural differences listed in the table, the two strains of each species were serologically distinct, and the

[3] The following salts were ground together and used in the proportion of 1½ gm. per liter of distilled water. After standing 24 hours, the clear solution was decanted and sterilized before use. KCl–100 gm., $CaSO_4 \cdot 2H_2O$—25 gm., $MgSO_4 \cdot 7H_2O$—25 gm., $Ca_3(PO_4)_2$—25 gm., K_2PO_4—25 gm.

agglutination reaction was used to differentiate the strains. The various differences between the two strains in each species were great enough to make differentiation positive and relatively easy.

Isolations

A summary of the isolations made from these plants is given in table 2. A typical plant was selected from each pot or jar, and six or more nodules were plated out from it using a yeast-water mannitol agar. Where inoculations had been made with the two strains, an attempt was made to select nodules characteristic of each type. The earlier experiments on strain variation (1, 5) have all called attention to the fact that with the ineffective strains the nodules are small and widely scattered over the root system, whereas with the effective

TABLE 2

Strains of Rhizobia recovered from nodules after double inoculation of alfalfa (Medicago sativa), red clover (Trifolium pratense), pea (Pisum sativum), and soybean (Soja max)

SERIES	INOCULATION PLAN*	ALFALFA	CLOVER	PEA	SOYBEAN
I	Control	.....	.....	.....	.....
II	Poor alone	Poor	Poor	Poor	Poor
III	Good alone	Good	Good	Good	Good
IV	Simultaneously good and poor	Good	Good and poor	Good and poor	Good and poor
V†	Poor and later good	Poor and good	Poor	Poor	Poor
VI†	Good and later poor	Good	Good and poor	Good	Good

* Strains used for inoculation are those listed in table 1.

† Second inoculation with alfalfa and clover 42 days after planting; with pea, 28 days; and with soybean, 30 days after planting.

strains the nodules are larger, fewer in number, and usually located on the upper portion of the root system. Although positive diagnosis of the effectiveness of a strain can not be made by the nodule size and type, a large proportion of such attempts by one familiar with the organisms and plants are successful.

The surface of the nodule was sterilized in a 1 to 500 mercuric chloride solution in order to remove the possibility of obtaining cultures from the sand rather than from the inside of the nodule. Not less than two colonies from each nodule were selected for study and identification, and wherever differences in colony characters were found several colonies were selected. In no instance was more than one strain found in any one nodule.

The results of this study show that a single individual of each of the host plants may under appropriate conditions of inoculation bear nodules formed both by effective and by ineffective strains of Rhizobia. Simultaneous inoculation with the two contrasting strains at the time of planting served to establish

both strains on the host plant, with clover, pea, and soybean, but not with alfalfa.

Although simultaneous inoculation of alfalfa at planting with the two strains of Rhizobia failed to give plants infected with both strains, inoculation with the effective strain 42 days after planting seed which had been inoculated with the ineffective strain, series V, gave plants bearing nodules formed by each strain. The reciprocal experiment, series VI, in which the good strain was used first, resulted in infection only with the good strain.

With clover, series IV, simultaneous inoculation with the good and poor strains, and series VI, inoculation first with the good strain and later with the poor strain gave infection with both strains of Rhizobia. In series V, where the poor strain was used for the primary inoculation and the good strain applied 42 days later, only the poor strain was recovered from the nodules.

With pea and soybean, only series IV, simultaneous inoculation with the two strains, served to give plants infected with the two strains. Pea and soybean are both annual plants, with a much shorter growth period than clover and alfalfa. It is possible that double infection might have been obtained in series V and VI had the second inoculation been applied at an earlier date.

Because of the realization that the failure to recover both strains from all plants to which the two strains were added might have been due to the element of chance in picking the nodules for study, another experiment was made in which a much larger number of nodules were studied. Clover was inoculated as before at planting with the ineffective strain and after 56 days a heavy inoculum of the effective strain was added to the jar. The plants were washed out when 174 days old and 100 nodules, comprising all the nodules on four of the side roots, were plated. The results here were the same as in the previous experiment—only the ineffective strain was recovered. It is characteristic of the ineffective strains to produce nodules well distributed over the root system, and many of the nodules plated in this study were located on roots which grew after the time of the second inoculation. Yet, only the ineffective strain was recovered from the 100 nodules studied.

Many other experiments will be necessary, using other strains of the organism and different methods of inoculation, before a complete analysis of the relation between the host and the lower organism can be given. Certain indications from these experiments, however, are worth noting. First, there is evidence, with clover, pea, and soybean, that the resistance of infected plants toward additional infection is greater against a new strain than against the one already within the plant. Second, the indications are that the infective power of the strains may vary independently of their effectiveness. With *Rhizobium meliloti*, the good strain, 100, is apparently more infective than the poor strain, 101, since strain 100 was recovered from all cases of double inoculation whereas strain 101 was recovered from series V only where it alone was present during the first 42 days of plant growth. Just the reverse is seen with *Rhizobium trifolii* where the poor strain, 202, appears to have greater infective power

than the good strain, 205. Here both strains were recovered where the good strain was applied first and later followed by the poor strain, but the good strain was not recovered when it was applied as the secondary inoculation.

Both of the foregoing hypotheses are dependent upon the assumption that the two strains of the organism were in contact with the root systems of the plant. An effort to accomplish this was made by the use of a very heavy inoculum which was immediately washed into the dry sand with a large volume of water. It is possible, but hardly probable, that antagonism between the two strains may have resulted in the rapid elimination of one of them.

EFFECT ON THE HOST OF DOUBLE INFECTION WITH GOOD AND POOR STRAINS OF RHIZOBIA

The data reported in the first part of this paper have demonstrated that under suitable conditions a leguminous plant may bear, simultaneously,

TABLE 3

*Effect on Pisum sativum of double infection with good and poor strains of Rhizobium leguminosarum**

SERIES	INOCULATION PLAN	STRAIN RECOVERED	DRY WEIGHTS	NITROGEN	
			gm.	*mgm.*	*per cent*
I	Uninoculated controls	.....	6.06	84.8	1.40
II	Poor	Poor	6.15	105.1	1.71
III	Good	Good	8.20	181.2	2.21
IV	Simultaneously poor and good	Poor and good	7.05	158.6	2.25
V	Poor, later good	Poor only	5.95	104.1	1.75
VI	Good, later poor	Good only	7.83	182.4	2.33

* The poor strain used was 310 and the good strain used was 313. Twelve plants in each series. Planted March 16. Harvested May 3. Second inoculation—28 days after planting.

nodules formed by effective and by ineffective strains of the nodule organism. The following experiments were designed to study the response of the host plant to such double infection.

Preliminary pea experiment

Dry weight and nitrogen contents are reported in table 3 for one of the experiments on peas from the preceding section. In this experiment, both the effective and the ineffective strains of the nodule organism were recovered from the plants which had been simultaneously inoculated with the two strains at planting time. The growth of the plants and the nitrogen content in this series were intermediate between that of the plants inoculated with the good strain alone and those inoculated with the poor strain alone.

When either the good or poor strain was applied first and followed after 28 days with the other, only the strain first applied was recovered. In both of these series, the plant growth and the percentage of nitrogen gave no evidence of any effect from the second inoculation.

Effect of time of inoculation

In the preceding experiment with peas, only the series in which the plants were inoculated at planting time with both strains bore nodules formed by the two strains. The failure to obtain entrance of the strain used for a second inoculation in series V and VI might have been due to the age of the plants at the time of the second inoculation. To test this point and to study further the response of plants to double infection with good and poor strains of the nodule organism, a set of experiments was started varying the time of inoculation and harvest.

In these experiments both pea (*Pisum sativum*) and red clover (*Trifolium pratense*) were used. The technic of manipulation was the same as that used in the earlier experiments except that all plants were grown in the half-gallon jars of sterilized sand. All possible precautions were taken to prevent contamination with other strains of Rhizobia.

At the end of approximately the first third of the growth period and again after about one-half of the growth period, part of the uninoculated controls and part of the plants nodulated with the poor strain, were inoculated with the good strain. The experiments were so arranged that part of the pots were washed out for analysis, (*a*) at the end of the first period, (*b*) at the end of the second period, and (*c*) at the completion of the experiment. Details of the inoculation are given as follows:

BEGINNING	END OF 1ST PERIOD	END OF SECOND PERIOD	END OF EXPERIMENT
Inoculation	Inoculation	Inoculation	
Controls	H*		
Controls		H	
Controls			H
Controls	Good	H	
Controls	Good		H
Controls		Good	H
Poor	H		
Poor		H	
Poor			H
Poor	Good	H	
Poor	Good		H
Poor		Good	H
Good	H		
Good		H	
Good			H

* The H indicates the period at which each set was harvested. Triplicate pots were carried in each case.

The data obtained in these experiments are recorded in table 4. The plants harvested at the end of the first period, with either red clover or peas, showed no effect of the inoculation although nodules were present on the roots. Evidently the seed nitrogen was adequate to carry the plants this far. At the end of the second period, the plants from seed which had been inoculated with the good strain at planting were much better than any of the others, with both peas and red clover. The corresponding sets inoculated with the poor strain had made

TABLE 4

Effect on the host plant of double infection with good and poor strains of Rhizobia

INOCULATION	ALASKA PEA*			RED CLOVER†		
	Dry weight	Nitrogen		Dry weight	Nitrogen	
Harvest at the end of the first period						
	gm.	*mgm.*	*per cent*	*gm.*	*mgm.*	*per cent*
Control	2.12	68.8	3.24	0.23	....	....
Poor strain	2.17	65.6	3.02	0.16	....	....
Good strain	2.03	58.7	2.89	0.21	....	....
Harvest at the end of the second period						
Control	4.76	85.2	1.79	0.71	7.5	1.05
Control plus good	4.30	82.1	1.91	0.62	8.4	1.34
Poor plus good	4.85	89.7	1.85	0.53	8.1	1.51
Poor	4.76	89.1	1.87	0.70	10.0	1.43
Good	5.51	166.9	3.03	1.47	38.7	2.57
Harvest at the end of experiment						
Control	5.85	95.9	1.64	0.81	12.6	1.55
Control plus good, early	8.36	211.5	2.53	5.54	110.3	1.99
Control plus good, late	4.88	61.9	1.27	2.67	55.6	2.08
Poor plus good, early	6.40	106.2	1.66	3.10	58.2	1.88
Poor plus good, late	7.13	112.6	1.58	2.91	53.9	1.85
Poor	5.93	97.8	1.65	1.16	24.0	2.06
Good	15.09	344.2	2.28	8.71	176.5	2.02

* Alaska pea inoculated with good strain, 313, and poor strain, 310. Plants 19 days old at end of first period, 31 days old at end of second, and 52 days old at end of experiment.

† Red clover inoculated with good strain, 205, and poor strain, 202; plants 38 days old at end of first period, 61 days old at end of second, and 123 days old at end of experiment.

essentially the same growth as the controls. No effect was apparent from the secondary inoculation with the good strain, either of control plants or plants already carrying the poor strain.

At the end of the experiment, however, definite differences were apparent in the growth and appearance of the various series. The dry weights and nitrogen contents of these plants are displayed graphically in figures 1 and 2, and plate 1 shows a photograph of the clover series at this time. With both clover

and peas, the plants from seed inoculated at planting time with the good strain were much the best, in total plant growth and in the amount of nitrogen in the plant tissue. With peas, the plants from seed inoculated with the poor strain at planting were essentially like the controls. With red clover, however, the series inoculated with the poor strain was slightly better than the control series.

Delayed inoculation with the good strain on previously uninoculated plants served to increase markedly both plant growth and nitrogen content with each host species. The early inoculation was much more effective than the late

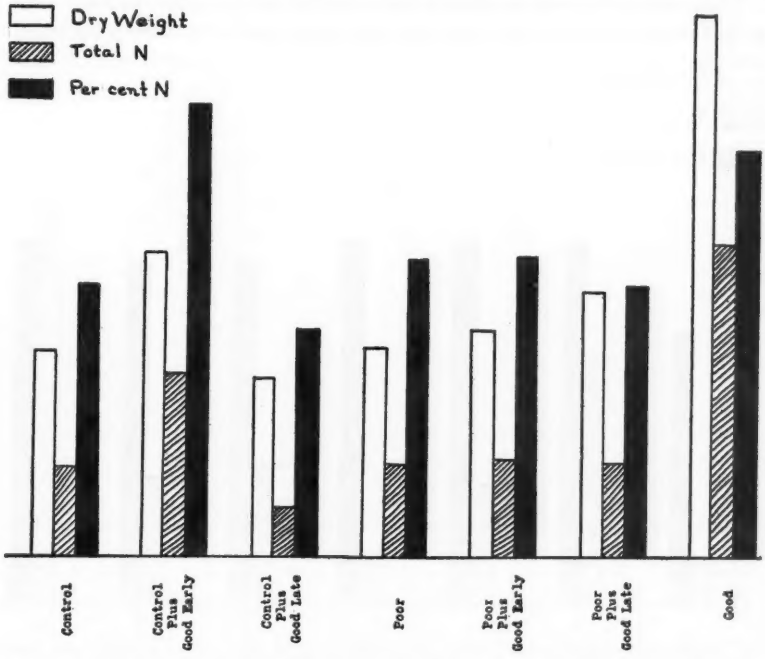

FIG. 1. THE EFFECT OF DOUBLE INFECTION WITH GOOD AND POOR STRAINS OF *Rhizobium leguminosarum* ON THE DRY WEIGHTS AND NITROGEN CONTENT OF PEAS (*Pisum sativum*)

inoculation in each case. Quite a different picture was presented where a secondary inoculation with the good strain was applied to plants already carrying the poor strain. The effect of the good strain in stimulating plant growth here was much less marked than where the good strain was applied under similar conditions to nodule-free plants. Also the time of applying the good strain seemed to be of less importance, as the plants receiving the late inoculation with the good strain finished the growth period with essentially the same dry weights and nitrogen contents as those receiving the earlier inoculation. This substantiates the finding of the isolation experiments; plants which

are nodule-free are much more easily infected with a good strain of Rhizobia than are plants which are already infected with a poor strain of the organism.

With peas, the dry weights of the plants at harvest are roughly proportional to the percentage of nitrogen. With red clover, however, there is little variation in the percentage of nitrogen in the plants of the different series, although the dry weights are quite different.

Comparison of natural versus artificial inoculation

The previous experiments have demonstrated that the earlier the good strain gained entrance into the plant the more the plant profited from the association.

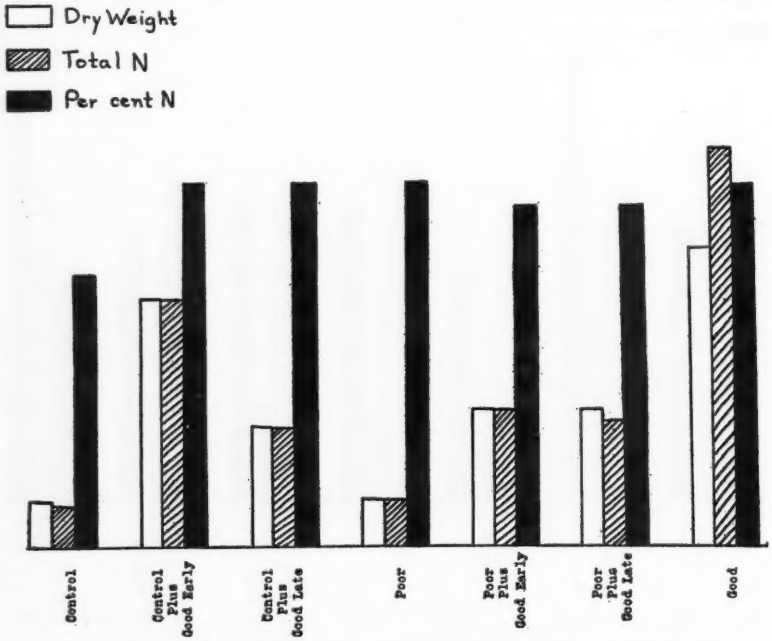

FIG. 2. THE EFFECT OF DOUBLE INFECTION WITH GOOD AND POOR STRAINS OF *Rhizobium trifolii* ON THE DRY WEIGHTS AND NITROGEN CONTENT OF RED CLOVER (*Trifolium pratense*)

This was particularly true in cases in which both organisms were present. Under field conditions either the good or poor strain may be present in soil in which inoculated seed is sown. Also experience with commercial cultures has demonstrated that not only good but also poor strains are at times used for seed inoculation. The following experiments were set up to determine the value of inoculating seed with an effective strain of the organisms and then planting it in soil carrying an ineffective strain; also to determine the effect of planting,

in a soil which carries an effective strain of the organisms, seed which had been inoculated with an ineffective strain.

In the first of these experiments, with peas and soybeans, sterile sand in half-gallon pots was inoculated with either the good or poor strain by adding 1 cc. of a heavy bacterial suspension of the organism and then bringing up to optimum moisture with sterile water. These inoculated pots were set aside for 3 days before planting. The seed were first rendered bacteria-free and then inoculated with the appropriate strains of the nodule organism. The inoculation plan and the data obtained from these experiments are presented in table 5.

With soybean when only one strain was used, essentially the same results were obtained regardless of whether the seed or the sand received the inoculum. With either host plant, those inoculated with the ineffective strain gave dry

TABLE 5

Comparison of soil versus seed inoculation of soybeans and peas with good and poor strains of Rhizobia

INOCULATION	SOYBEAN*			PEA†		
	Dry weight	Nitrogen		Dry weight	Nitrogen	
	gm.	*mgm.*	*per cent*	*gm.*	*mgm.*	*per cent*
Control	9.6	114.2	1.18	3.9	126.7	3.25
Sand, good strain	12.9	227.2	1.76	...	....	....
Sand, poor strain	9.2	107.6	1.17	...	....	....
Seed, good strain	11.5	206.2	1.79	5.1	175.7	3.44
Seed, poor strain	9.7	115.4	1.19	4.3	133.0	3.08
Sand, poor strain; seed, good strain	10.9	205.2	1.88	4.8	170.6	3.49
Sand, good strain; seed, poor strain	12.5	250.5	1.95	5.2	176.5	3.35

* Soybean inoculated with good strain, 501, and poor strain, 507. Planted May 21. Harvested July 6. Three pots with four plants each.

† Pea inoculated with good strain, 313, and poor strain310. Planted September 26. Harvested November 23. Three pots with four plants each.

weights and nitrogen percentages which were approximately the same as the control plants. Inoculation of plants with the effective strain markedly increased the plant growth and nitrogen content of the plants.

Under the conditions of these experiments with either host, the effective strain seemed to dominate over the ineffective strain, regardless of which was applied to the seed and which was present in the soil. It was noticeable, however, that the strain which was in the sand seemed to be slightly more active. This was probably due to the larger numbers present in the sand. Where the sand carried the good strain and the seed the poor strain, the plants made just as good growth as the plants in those pots containing only the good strain. Slightly poorer plant growth resulted from the planting of seed inoculated with the good strain in sand carrying the poor strain.

In these experiments, conditions as they might exist in the field were only

approximated, since the inoculum applied to the sand was much heavier than would be found under ordinary soil conditions. To approach normal soil conditions more nearly, a second experiment was started, in which the sand inoculation was that residual from a first crop.

A number of pots of sterilized sand were planted with peas (*Pisum sativum*), part of which had been inoculated with an effective strain, 313, and part with an ineffective strain, 310. At maturity the tops of these plants were harvested and the pots with undisturbed root systems were thoroughly moistened with sterile water, covered with sterile paper, and set aside for 3 weeks. Although sterile sand was used at the start, exposure to the greenhouse air resulted in the

TABLE 6

Comparison of natural versus seed inoculation of pea (Pisum sativum) with good and poor strains of Rhizobia

STRAIN OF FIRST PLANTING*	RE-INOCULATION, SECOND PLANTING*	COLOR	NODULES	DRY WEIGHT	NITROGEN	
First crop—tops only						
				gm.	*mgm.*	*per cent*
Poor	.....	Pale green	Small scattered	4.05	76.9	1.90
Good	.....	Rich green	Large on tap root	6.30	178.9	2.84
Second crop—tops and roots						
Poor	0	Pale, stunted	Many small	4.49	134.7	3.00
Poor	Poor	Pale	Small and medium, scattered	4.86	148.7	3.06
Poor	Good	Good green	Small and medium, of both types	5.08	160.5	3.16
Good	0	Good green	Medium to large	6.01	204.3	3.40
Good	Poor	Medium green	Medium to small	4.48	147.8	3.30
Good	Good	Good green	Medium to large, good type	6.02	201.6	3.35

* Pots in triplicate with 6 plants in a pot—18 plants. Three-week interval between the harvest of the first crop and the planting of the second.

entry of many types of organisms and the roots partially decayed in the 3-week rest period. These plants had been grown in a separate greenhouse and control plants did not show nodules, indicating that little if any contamination of the pots with nodule organisms had occurred. For the second planting, bacteria-free seed were inoculated either with the good or poor strain and planted in these pots.

The inoculation plan and the data obtained were presented in table 6. Seed reinoculated with the homologous strain, i.e., the strain already present in the pots, gave essentially the same plant growth as did seed that was not inoculated. Inoculation of seed with the heterologous strain, i.e., the contrasting strain, however, gave marked results. When planted in the sand carrying the

poor strain, seed inoculated with the good strain gave improved plant growth in comparison with either uninoculated seed or seed inoculated with the poor strain. On the other hand, with the sand carrying the good strain, inoculation of the seed with the poor strain reduced plant growth in comparison with either uninoculated seed or seed carrying the good strain.

It is interesting to note that all of the plants in the second planting bore many nodules, and that the type of nodules corresponded well with the plant growth. In the sand carrying the poor strain, only the plants from seed inoculated with the good strain bore large nodules. All the others bore many small nodules. In the other series only the plants from seed inoculated with the ineffective strain bore the numerous scattered nodules characteristic of the ineffective strain, whereas the others had fewer but larger nodules.

Under these conditions which more nearly approximate field conditions, it is apparent that seed inoculation with an effective strain may materially improve plant growth where the soil already contains many nodule-forming organisms of an ineffective type. Also, inoculation of seed with an ineffective strain of the organism may be detrimental to crop growth if the soil already contains an effective strain of the organism.

SUMMARY

Isolation studies from leguminous plants, clover, alfalfa, pea, and soybean, inoculated with both effective and ineffective strains of the nodule organisms show that a single plant may bear at one time nodules formed by each of the strains. In no instance were two strains found in a single nodule.

The evidence from these studies indicates that the strains of the nodule organism may vary in their infective power independently of their ability to aid the growth of the host plant, since certain strains seemed to be able to enter the plant more readily than others.

Plants already infected with one strain of the nodule organism resist the entrance of a contrasting strain to a much greater extent than do nodule-free plants. On the other hand, nodulated plants are apparently open to further infection by the homologous strain of the nodule organism as long as new roots are formed. This is particularly true of plants bearing the ineffective strain, where the nitrogen supply of the plant is deficient

Plants bearing both an effective and an ineffective strain of the nodule organism give growth and nitrogen fixation intermediate between those bearing either strain alone.

The earlier the effective strain gains entrance into the plant the more benefit the plant derives from the association This effect is more pronounced with plants which were previously nodule-free than with plants already infected by the poor strain.

Improved plant growth results from inoculation of seed with an effective strain when it is sown in soil carrying an ineffective strain. On the other hand, inoculation of seed with an ineffective strain is detrimental to plant growth, if the seed is planted on soil already carrying an effective strain of the organism.

The results of these studies indicate the necessity of using for seed inoculation only effective strains of the nodule organism, since definite detrimental results may occur from the use of ineffective strains.

It is probable that many of the favorable results obtained from seed inoculation on old land are due to the introduction of more effective strains of the nodule organism. Also that many of the failures to produce increased plant growth by seed inoculation are due to the use of ineffective strains of the organism.

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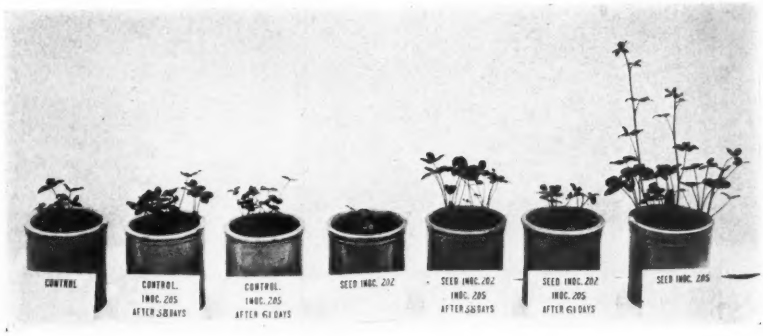
PLATE 1

Red Clover Plants Just Before Harvest (123 Days Old) Showing the Growth Following Inoculation at Various Times


CONTROL
CONTROL. INOC. 205 AFTER 58 DAYS
CONTROL. INOC. 205 AFTER 61 DAYS
SEED INOC. 202
SEED INOC. 202 INOC. 205 AFTER 58 DAYS
SEED INOC. 202 INOC. 205 AFTER 61 DAYS
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